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APPLICATIONS OF MICROBIAL TECHNOLOGY

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CHAPTER 1

MICROBIAL APPLICATIONS IN AGRICULTURE AND THE ENVIRONMENT

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ABSTRACT:

A significant part of the total global biological variety is the diversity of microbes. Recent technical developments in microbial diversity exploration have shown that many microorganisms remain unexplored, and their ecological functions are mainly unknown. The main stages in creating new technologies for the efficient use of microorganisms for sustainable agriculture, environmental protection, and human and animal health are careful selection of bacteria and clever test assay design. Numerous microbial uses are well recognised for resolving significant agricultural and environmental problems. Other significant applications of microbial technology include wastewater treatment and recycling of industrial and agricultural waste. Microbes are predicted to play a big part in addressing 21st-century global issues, such as climate change, in conjunction with advancements in electronics, software, digital imagery, and nanotechnology. It is anticipated that these developments would improve environmental and agricultural sustainability. This chapter offers a summary of current developments in the use of microorganisms to promote plant growth and a sustainable environment, mostly via bioremediation, biodegradation, and biosorption procedures. Probiotics, nanomaterial production, biosensors, and other recent uses and applications of microorganisms are also covered.

KEYWORDS: Agriculture, Biosensors, Climate, Environment, Microbial.

1. INTRODUCTION

The biggest untapped source of biodiversity on Earth is the microbial world. It is a crucial biological area that is now the subject of much research. The fact that bacteria carry out a variety of vital tasks for the biosphere, including as nutrient cycle and environmental detoxification, has sparked interest in the study of microbial diversity. Strong foundations for understanding microbial variety, conservation, and exploitation for society are provided by their enormous range of activities and by their significance to the biosphere and to human economy[1].

In recent years, it has become rather evident what the environmental "super challenges" of the twenty-first century would entail. There is actual climate change as a result of the enormous rise in greenhouse gas output. Renewable energy sources are really needed. Water shortages, declining agricultural output, environmental degradation, and ongoing pandemic threats like the Asian flu, Mad Cow disease, and Legionella epidemic are a few of the crucial problems. What solutions can microbial resources provide to these problems? There are many different kinds of microorganisms in the soil, water, air, and in close proximity to plants and animals.

These many groups make up "a metagenome of knowledge." Our body's internal and external microbial populations are included in this metagenome. They have a significant role in environmental sustainability as well as human health and well-being because of their metabolic activities[2].

More than a million distinct strains of microorganisms may today be found in microbial culture collections, which is evidence of the efforts undertaken to preserve biodiversity and make these resources accessible to the general population. It is debated whether or not these collections should or should not be enlarged. It is well acknowledged that germs often function in groups rather than on their own. It follows that significant effort should be put forward right now to gather and preserve these unique microbial interactions in both enrichment cultures and wild samples. The ecosystems in which these bacteria flourish must also be preserved. Up until now, the majority of the emphasis has been on different unusual areas, such hot springs and beautiful landscapes. For instance, the latter has resulted in a wealth of information about unique polar microbial taxa during the last ten years, which has in turn led to the development of commercial applications such as cold-adapted enzymes, anti-freeze products, and several additional strains capable of bioremediation in cold soils.

New frontier habitats like the deep seas, the deep earth, endophytic microorganisms, and the deep gut need to be explored more thoroughly. Such surroundings do indeed include a multitude of allegedly beneficial processes and goods. The conversion of ammonium and nitrite to dinitrogen gas by anaerobic ammonium oxidation, the Archaea-Bacteria consortia that anaerobically oxidise methane through the use of sulphate reduction, and the pH-tolerant humus-degrading bacteria found in the gut of soil-eating termites are just a few of the recent fascinating discoveries. In addition to these "natural" habitats, a number of additional locations that have undergone industrial alteration and are often unwelcome are now to be designated as "resources" of microbial variety. The finest examples of these resources include aquifers polluted with chloroorganics, which have produced highly fascinating halo-respiring microorganisms, and areas subjected to acid mine drainage, which have lately supplied promising anticancer medications. The choice of certain unknown microbial habitats, whether they be natural or manmade, might thus have major implications for the environment, agriculture, and civilization[3].

Methods for Analysing Soil Microbe Populations

It is believed that soil is a repository for microbial activity. It is believed that fewer than 5% of the overall volume is inhabited by living bacteria. As a result, the majority of microbial activity is restricted to "hot spots," or groups of accumulated organic matter, such the rhizosphere. Because of their enormous phenotypic and genotypic variety, variability, and crypticity, soil microbial communities are sometimes challenging to characterise. Regarding the latter, bacterial populations may generate more than 9 cells/g of soil in the upper soil layers. Many of these cells cannot be cultured. Less than 5% of the total population of the cells that make up soil microbial biomass have been extensively investigated and are considered to be inconsequential. Different methods may be used to investigate the variety of the soil's microbes, biochemical reactions, and functional components.The two primary types of microbial diversity research techniques are cultivation-based and cultivation-independent techniques. Both strategies have certain drawbacks and benefits.

Plant-Based Techniques

The culture and isolation of microorganisms served as the foundation for traditional approaches to studying microbial diversity. To increase the diversity and populations of microorganisms, a broad range of culture mediums have been developed. Microbial diversity

has been investigated using a BiologTM-based approach for directly analysing the potential activity of soil microbial communities presenting community level physiological profile.

Methods Independent of Cultivation

Understanding the variety of soil microbes has been made easier by recent advances in molecular technology. These molecular methods, which are used to target the particular DNA or RNA in soil, include polymerase chain reaction and real-time polymerase chain reaction. Useful indicators for prokaryotes and eukaryotes, respectively, are the S or S ribosomal RNA or their genes. When whole DNA or RNA from a particular soil microbial community is used to create PCR products, primers based on conserved sections of the S or S rDNA are used. This results in a combination of DNA fragments representing every PCR accessible species present in the soil sample. For creating clone libraries and a variety of microbial community fingerprinting, you may utilise the mixed PCR products. These clone libraries may be used to identify and characterise the predominant bacterial or fungal species in soil and so provide an overview of the microbial diversity present there. The fingerprinting of soil microbial communities has also been done using a variety of different techniques. Examples of techniques that have been used successfully include denaturing or temperature gradient gel electrophoresis, amplified rDNA restriction analysis, terminal restriction fragment length polymorphism, single-stranded conformational polymorphism, and ribosomal intergenic spacer analysis.

Microbes' Functional Diversity

The most crucial factor for microbial culture exploitation and characterization is functional diversity. Similar to this, functional genomics is regarded as a potent method for identifying new functions linked to an organism's DNA. They have been given many names that reflect their primary roles in nature or under certain circumstances, depending on the organism's intended usage. However, a good screening technique requires sophisticated test system design and careful microbe selection in order to get a unique class of chemicals and activities. To enhance the production and/or fitness of the culture in a particular site, strain improvement programmes may also be applied to the wild strain derived from diverse reservoirs[4].

DISCUSSION

Environmental and Agricultural Applications

Numerous soil and other sources' microorganisms have been extensively investigated and used for composting, crop protection, soil health improvement, and crop production. Additionally, microbial products have been used to manage plant and animal illnesses. Transgenic plants with enhanced gene delivery mechanisms are now conceivable because to recent advancements in microbial and plant molecular biology. Transgenic crop success stories are now widely available. However, the use of microbes in the food, medicinal, and industrial sectors is extensive and beyond the purview of this paper. Similar to this, environmental exploitation for bioremediation of contaminated soil, water, and other ecosystems is widely recognised and well-documented in the literature. However, new ways are required to discover and use the latent microbial variety in agriculture, the environment, and human health owing to a lack of information on the microbial diversity of diverse distinct harsh environments and a poor understanding of nonculturable bacteria. In addition to the traditional applications and uses of microorganisms, additional dimensions have been investigated where it is anticipated that bacteria will provide answers to particular issues and applications. Here, some of them are briefly covered[5].

Plant Growth Promotion and Health Protection by Microbes

Throughout the globe, plant-pathogenic microbes pose a serious and ongoing danger to the stability of ecosystems and food supply. Producers have relied more and more on agro-chemicals as a reasonably dependable means of crop protection as agricultural output has increased over the last several decades, which eventually brings economic stability to their operations. However, the increased usage of chemical inputs has a number of unfavourable consequences. The principles of integrated plant nutrition management and integrated plant disease and pest control must now be improved in accordance with available resources, agroclimatic conditions, and economic factors. Utilising biological agents is a crucial component of management in this strategy.

Thus, biocontrol is being considered as an additional or alternative method of lowering the usage of pesticides in agriculture. A substantial amount of research has been done on the possible use of bacteria found in plants for regulating soil and plant health, stimulating plants, and other purposes. By Kloepper and Scroth in, the phrase "plant growth-promoting bacteria" was first used. The majority of PGPRs are fluorescent Pseudomonas. PGPB and plant growth-promoting fungi, which may be symbiotic or free-living in the rhizosphere, are connected to most, if not all, plant species and can be found in a variety of habitats. Plant growth-promoting rhizobacteria, which populate root surfaces and the tightly adherent soil interface known as the rhizosphere, are the most extensively researched group of PGPBs. These PGPR might be free-living, endophytic, diazotrophic, non-diazotrophic, or include other symbiotic fungi[6].

Competition for an ecological niche or a substrate, the generation of inhibitory allochemicals, and the creation of systemic resistance in host plants to a wide range of diseases and/or abiotic stressors are some of the well acknowledged mechanisms of biocontrol mediated by PGPBs. The many features of disease suppression by these biocontrol agents are now well understood because to research into the processes of plant growth promotion by PGPB. However, free-living rhizobacterial species, particularly Pseudomonas and Bacillus, have received the majority of attention. There is still much to learn about nonsymbiotic endophytic bacteria, which have unusual relationships and seem to have a more noticeable growth-stimulating impact on host plants. The mechanisms of PGPB activity have been revealed, opening up new avenues for the development of biocontrol agents with increased effectiveness.

Similar to how soil aeration, hydrogeology, and supply of molasses, sugars, and proper crop rotations may affect bacterial consortia in the rhizosphere. The ability to combine strains of bacteria with bacteria or bacteria with fungus to attack pathogens with a wider variety of microbial weapons is made possible by the identification of several mechanisms of action. In a similar vein, transgenic strains that combine several modes of action may be developed in order to further enhance strains that already have desirable features. For instance, changing the 1-aminocyclopropane-1- carboxylic acid deaminase gene may boost the biocontrol capabilities of PGPB while also directly stimulating plant growth by cleaving the immediate precursor of plant ethylene into P. fluorescens CHAO. As with the associative nitrogen-fixing PGPB on sugarcane or the nonsymbiotic endophyte bacterium Burkholderia phytofirmans, further work with endophytic bacteria may lead to the development of biocontrol agents that may be self-perpetuating by colonising hosts and being passed on to offspring. For efficient use, a new microbial agent's performance should be evaluated in integrated plant nutrition management[7].

Acetobacter, Azotobacter, Azospirillum, Burkholderia, and Bacilli species are among the additional bacteria that are currently thought to be PGPR. Almost any free-living, non-deleterious bacterium that could directly or indirectly encourage plant development may be classified as PGPR. Achromobacter, Arthobacter, Azocarus, Clostridium, Enterobacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleous, Phyllobacterium, Serratia, Staphylococcus, Streptomyces, and Vibrio are only a few of the additional PGPR that have recently been discovered.

PGPR may, directly or indirectly, encourage plant growth. The capacity to create plant growth regulators, asymbiotic N2 fixation, and solubilization of mineral nutrients like phosphates are examples of direct mechanisms. According to Ahmad and Ahmad et al., indirect mechanisms include antagonistic effects on phytopathogens, the synthesis of siderophores, the development of extracellular cell wall-degrading enzymes produced by phytopathogens such -1, 3-glucanase and chitinase, the production of antibiotics, and the generation of cyanide.

Fungi that Promote Plant Growth

Numerous plant-associated microorganisms play important roles; examples include the relationship between rhizobium and legumes and the function of mycorrhiza in promoting plant development. Through a variety of mechanisms, free-living fungi have also been linked to the stimulation of plant development. Phosphate solubilization is a prime illustration of this. Phosphate solubilizers are the common name for these organisms. In India and other areas of the globe, the -s saw extensive research into the function of these organ-isms. However, fresh interest has been displayed by many professionals as a result of the discovery of additional environmental advantages connected with these species. Imran recently discovered that many phosphate-solubilizing fungi have a variety of additional beneficial traits. These traits include the ability to produce plant growth hormones, numerous extracellular enzymes, resistance to many toxic metals, the ability to biosorb Ni, Cd, and Cr, as well as a contribution to plant growth enhancement when used as inoculants for wheat and other crops. In order to properly use these species for managing environmental pollution and agricultural production, more creatures should be explored[8].

Environmental Problem Management using Microbes

Globally, environmental contaminants in soil and water are a serious problem. The environment and public health are seriously threatened by a number of poisonous, mutagenic, and carcinogenic substances. Chemical, physical, and biological methods may be used to treat contaminated water and wastewater in order to eliminate and/or detoxify it. Similar techniques may be used to remediate polluted soil, including thermal desorption and landfilling. However, these soil improvements do not successfully reestablish the native vegetation and animals. The most promising method is bioremediation, which uses microorganisms to eliminate dangerous chemicals from the environment. It is eco-friendly, safe, and efficient even when the contaminants are present in small amounts.

Many site-specific microorganisms have already been applied to sites that were previously polluted with polycyclic aromatic hydrocarbons, nitroaromatic chemicals, chlorinated organics, etc. They are capable of carrying out bioremediation activities. The pollutants' derivatives may build up and cause their own particular health problems since they are often not fully mineralized. Numerous approaches are being examined to address this issue, including the employment of different combinations of microbes that have the capacity to mineralize certain forms of the contaminants and their derivatives. The potential and possibilities of microflora in the quick breakdown of pesticides like benzene hexachloride

and similar chemicals were mentioned in a superb review study by Lal et al. Exploration and/or creation of novel catabolic pathways as well as research into the regulatory control of primary and secondary metabolites are other critical directions that must be taken in this area in order to produce efficient bioremediation reactions. This is a challenging undertaking because we lack comprehensive knowledge of how in situ bacterial adaptability to environmental stressors and regulation of different metabolic genes works. However, it is now feasible to investigate global protein expression and low molecular weight metabolite expression in environmental bioremediation because to advances in bacterial genomes, proteomics, and metabolomics, as well as the development of sophisticated new tools in the medical sciences.

Utilising organisms or products derived from organisms to breakdown contaminants is known as bioremediation. The main benefit of bioremediation is that it is less expensive than traditional methods like incineration, which require remediating all polluted sites in the USA alone at a cost of $1.7 trillion, or $7,0 per resident. Additionally, bioremediation often offers a long-term remedy as opposed to a technique that only moves wastes from one stage to another. The catabolic potential of biological catalysts for the remediation of wastes is immense; nevertheless, because of the often intricate interactions between bacteria and contaminants, effective remediation is not always achieved. Additionally, a lot of man-made materials lack effective biological catalysts. In light of this, the sector is still fruitful for the use of novel biotechnological techniques to aid in bioremediation, such as metabolic engineering, proteomics, reverse genetics, transcriptomics, metabolomics, and genome-scale metabolic modelling. Follow-up studies are crucial for figuring out why contaminants continue to exist. Recombinant engineering is used in metabolic engineering to reroute a cell's metabolism towards a certain objective.

The metabolic engineering of Pseudomonas sp. B, in which five separate catabolic pathways from three different bacteria were combined to enable the breakdown of methylphenols and methylbenzoates in a single organism, was one of the earliest and best instances of this method in bioremediation. Ju and Parales developed the first strain of bacteria that can thrive on 3-chloronitrobenzene and made it possible for bacteria to use chloronitrobenzenes for development without the need for cosubstrates. For the production of insecticides, fungicides, dyes, and polymers, chloronitrobenzenes are produced. By slyly introducing nitrobenzene 1,2-dioxygenase from Comamonas sp. strain JS5 into Ralstonia sp. strain JS5, a strain that has an ortho route for the breakdown of chloro-catechols, the bacteria manage to achieve this feat. The authors painstakingly demonstrate how the cloned nitrobenzene 1,2-dioxygenase transforms 3-chloronitrobenzene into 4-chlorocatechol, which is then broken down by the host Ralstonia sp. strain JS5. They also use a large subunit dioxygenase active-site mutant to shorten the doubling time on 3-chloronitrobenzene.

CONCLUSION

The paper by Fernandez et al. demonstrates that the model bacterium Pseudomonas putida KT may thrive in the presence of saturated concentrations of the commonly used nitroaromatic explosive, 2,4,6-trinitrotoluene, which is related to the breakdown of nitroaromatic chemicals by bacteria. The scientists discovered that the organism responds to the molecule by activating a number of detoxification processes, including nitroreductase, isoquinolone oxidoreductase, dehydrogenase, and chaperones in order to avoid or repair cell damage. The authors also demonstrate how reducing intracellular trinitrotoluene concentrations induces the expression of multidrug efflux pump genes. This research is remarkable since it is the first time that transcriptomics has been used to study bioremediation. Transcriptomics has the potential to help solve previously unrecognised

regulatory bottlenecks that hinder remediation performance. In order to identify mutualistic interactions in the rhizosphere for strains important for bioremediation, such as rhizosphere, Matilla et al. employed whole-transcriptome profiling.

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CHAPTER 2

MICROBES IN METAL REMOVAL FROM WATER

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ABSTRACT:

The presence of heavy metals in water is a growing concern due to their toxic effects on the environment and human health. Microbial processes have emerged as a promising strategy for removing heavy metals from water due to their efficiency, cost-effectiveness, and environmentally friendly nature. In this context, various types of microbes, including bacteria, fungi, and algae, have been explored for their metal-removing capabilities. The present review summarizes recent advances in the use of microbes for heavy metal removal from water, highlighting the mechanisms underlying metal removal, factors affecting microbial activity, and the potential of combining microbial processes with physical and chemical treatments to enhance metal removal efficiency. The review also discusses the challenges and future prospects of using microbes in metal removal from water, emphasizing the need for further research to optimize microbial processes and scale them up for practical applications.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Scheibe et al. offer a genome-based metabolic model of the metabolism of Geobacter sulfurreducens and connect it with a hydrological transport model to predict in situ uranium bioremediation as another example of subsurface pollution. Predictive modelling definitely aids in identifying the limiting parameters and concentrations under natural environmental settings since the capacity of Geobacter to decrease U is vitally reliant on the availability of acetate as an electron donor and Fe as an electron acceptor. The effectiveness of combining genome-scale metabolic models with hydrological models for field-scale behaviour was shown by the model's ability to predict Geobacter behaviour in a field experiment of uranium bioremediation. At two fuel-contaminated sites, Gieg et al. give more insights on the rates of intrinsic bioremediation, or the microbial breakdown of hydrocarbon subsurface contaminants under anaerobic circumstances. Since many laboratory experiments measure lags of hours to days for a wide range of compounds, they demonstrate using deuterated compounds and skillful analytical work that the long lag phases seen in many of these experiments may not adequately predict the fate of these fuel contaminants. As a result, these pollutants may be degraded much more quickly than predicted. Identification of degradation intermediates involving fumarate as well as other intermediates provides evidence that a variety of chemicals, including as toluene, m-xylene, ethyl-benzene, 1,3,5,-trimethylbenzene, and hexane, may be remedied using anaerobic bioprocesses[1].

PAH Degradation In the environmental microbiology literature, PAHs with more than three aromatic rings are referred to as high molecular weight PAHs. HMW PAHs have physical

and chemical characteristics that make them seem to remain in the environment and may be harmful to human and ecological health in their parent molecule form or after undergoing biological and/or chemical transformations. HMW PAHs are electrochemically stable, have a limited solubility in water, and, depending on the conditions and route of exposure, may be acutely toxic, genotoxic, immunotoxic, or function as agents of hormone disruption. HMW PAHs may partition into organic phases, soil and sediment organic matter, and membranes of living organisms due to their high octanol-water partition coefficients. As a result, they are candidates for bioconcentration, bioaccumulation, and occasionally biomagnification through trophic transfers in terrestrial and marine food webs. HMW PAHs have been examined in the atmosphere, soil, freshwater and marine sediments, ice cores, deep seas, and several other media ranging from vegetation to food. Their quantities in the environment vary greatly, and they seem to be ubiquitous.

Prokaryotic biotransformation of HMW PAHs has drawn increasing attention, and investigations in this area are being conducted by teams of researchers from all over the globe, according to the scientific literature. Through the recording of novel isolates that represent various bacterial species that have been isolated from various settings and that show various metabolic capacities, research on the biodegradation of HMW PAH by bacteria has improved in recent years. Along with the continuing of thorough, in-depth characterizations of previously isolated organisms like Mycobacterium vanbaalenii PYR-1, this has taken place.

Characterization of new metabolites produced by the bacterial biodegradation of four- and five-ring PAHs. The understanding of the enzymes involved in these transformations has increased, and the routes for HMW PAH biodegradation have been improved, broadened, and further explored. Our knowledge of the ability of microbes functioning as communities during HMW PAH has also been advanced by research on prokaryotic consortia[2].

Water Microbe Removal from Metal

The use of microbe-based sorbents for the removal and recovery of strategic and valuable heavy metals from industrial wastewater is a new advancement in environmental microbial technology. Numerous microorganisms, including bacteria, fungus, algae, and yeast, have come under close investigation for their ability to actively and passively extract heavy metals from aqueous solutions. The biosorption process may be able to replace traditional heavy metal pollution management methods, or at the at least, may be more efficient when used in tandem with other methods for bioremediation, according to advancements achieved in the previous two decades.

PGPR in Metal Toxicity Biomanagement

Soil, groundwater, and marine ecosystems have all been heavily contaminated as a consequence of incorrect disposal, abuse, and inadvertent releases of harmful organic and inorganic substances into the environment. As the harmful consequences of these compounds on the environment and human health come to light, more focus is being placed on the creation and use of cutting-edge technologies for cleaning up this pollution. Heavy metal contamination of agricultural soil has been rising, mostly as a result of inadequately treated wastewater and sewage disposal as well as agricultural runoff in many developing and industrialised nations. Such pollution has had a negative impact on agricultural yield as well as soil health. To lessen the toxicity of metals to plants, PGPB may be helpful. There are two ways this event might happen. Plants grown in soil enriched with metals could experience less stress ethylene if PGPB with ACC deaminase is used. Additionally, complexes of iron and bacterial siderophores may be absorbed and used by plants. A plant cannot acquire

enough iron in metal-contaminated soils without the presence of bacterial siderophores because plant siderophores have a far lower affinity for binding to iron than do bacterial siderophores[3].

Kluyvera ascorbata SUD5, a bacteria that stimulates plant development and was isolated from soil gathered around Sudbury, Ontario, Canada, had significant concentrations of heavy metals. The bacteria had resistance to the harmful effects of Ni2+, Pb2+, Zn2+, and CrO. It also generated a siderophore and had activity for the deamination of 1-aminocyclopropane-1-carboxylic acid. Inoculated with this bacteria and cultivated in gnotobiotic conditions with high levels of nickel chloride, canola seeds were partly protected against nickel toxicity. In addition, in pot trials with canola and tomato seeds, the bacterium's resistance to nickel toxicity was clearly visible. The quantity of nickel that accumulated per milligramme of either the roots or shoots of canola plants was unaffected by the presence of K. ascorbata SUD5. Therefore, it seems unlikely that the decrease in nickel absorption by seedlings was the cause of the bacterial plant growth-promoting impact in nickel-containing environments. Instead, it could demonstrate the bacterium's capacity to reduce the amount of stress ethylene brought on by the nickel.

In comparison to the wild-type bacterium K. ascorbata SUD 5, the siderophore-overproducing mutant K. ascorbata 5/ had a more noticeable impact on plant growth. It was hypothesised that this capacity of these bacteria to shield plants from the inhibitory effects of high concentrations of nickel, lead, and zinc was due to the bacteria's ability to provide plants with enough iron.

In a long-term tannery waste-contaminated soil, Rajkumar et al. recovered RNP4, which they then characterised and assumed to be Pseudomonas sp. On 6+ Luria-Britani agar medium, the strain RP4 tolerated doses of up to 0 mg/L of Cr and significantly reduced Cr6+ to Cr3+. Additionally, in the presence of Cr6+, the strain was able to encourage the development of black gramme, Indian mustard, and pearl millet. Thus, the simultaneous bioremediation and plant growth promotion abilities of two new isolates were important for managing environmental and agricultural issues[4].

DISCUSSION

Microbial Biosensors and their Applications

A biosensor is a kind of analytical tool that combines a transducer with a biological sensing component to generate a signal proportional to analyte concentration. This signal might be the consequence of cellular metabolism-induced changes in proton concentration, gas release or uptake, light emission, absorption, and other processes. This biological signal is transformed by the transducer into a quantifiable response, such as current, potential, or light absorption, that may be amplified, processed, and recorded for subsequent examination. Biosensors may be made from a variety of biological components, such as enzymes, antibodies, receptors, organelles, and microbes. Additionally, tissue or cells from higher species, such as those from plants and animals, have been utilised. The usage of biological sources derived from microbes is more prevalent among them. To build biosensor devices, microorganisms have been combined with a range of transducers, including amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescent, and fluorescent. There is a lot of published scientific material on the development of microbial biosensors.

The most often utilised biological sensing component in the construction of biosensors is an enzyme; although highly selective, purifying an enzyme is an expensive and time-consuming operation. Microorganisms provide a perfect substitute, but they lack the enzymes' level of

specificity. Recent developments in molecular biology and RDT have created new opportunities for customising microorganisms to increase an enzyme's activity or express foreign enzymes/proteins in a host cell. The intimate contact between microorganisms and the transducer serves as the foundation of a microbial biosensor. Therefore, immobilisation on a nearby transducer is necessary for the creation of a microbial biosensor. As a result, immobilisation technology is crucial. It is possible to immobilise the microorganism on transducer or support matrices using a variety of physical and chemical techniques. You may read about these strategies in detail in the literature, along with their benefits and drawbacks. Based on the transducers, microbial biosensors may be divided into electrochemical, optical, and other types.

For use in environmental, food, military, and medicinal applications, several biosensors have been created. This is mostly because to its affordability, durability, and broad variety of pH and temperature operating ranges. The development of highly effective microbial sensors is currently constrained by their slower reaction times, lower sensitivity, and worse selectivity when compared to enzyme biosensors. There is potential for the future development of microbial biosensors for harsh environments and with more specialised applications thanks to recent advances in microbial genomics and DNA technology.

Nanoppapers and Microorganisms

The cutting edge of the rapidly expanding area of nanotechnology is represented by nanomaterials. The vast field of microbial or bionanotechnology encompasses the use of bacteria in the synthesis of nanoppapers. Experts from physics, chemistry, biology, and engineering must work together in this multi-disciplinary field. Due of the special optical, chemical, and electrical characteristics of nanomaterials, there is a great deal of interest in their creation. In fields including catalysis, biomedicine, mechanics, magnetics, and energy sciences, nanotechnology will play a crucial role in the new millennium thanks to recent advancements in the organisation of nanoscale structures into predetermined super-structures.

In materials science, it is still difficult to synthesise nanoppapers with high monodispersity and a variety of chemical compositions. There are a number of industrial processes in use that often include processing atomistic, molecular, and particulate matter in a vacuum or a liquid medium. The majority of procedures waste a lot of resources and utilise energy and materials inefficiently. Therefore, the demand to create safe, non-toxic, and environmentally friendly synthesis processes is always increasing. As a result, scientists studying the creation of nanoppapers have looked to biological processes for inspiration. Numerous species have the ability to produce inorganic compounds either intracellularly or extracellularly, as is widely known. For instance, diatoms synthesise siliceous materials, while unicellular organisms like magnetotactic bacteria generate magnetite nanoppapers. Multicellular organisms use inorganic elements as a basis to create complex structures, producing hard inorganic-organic composite materials like bones, shells, and spicules[5].

A particular organic matrix that regulates the morphology of the inorganic substance makes up the inorganic component of biominerals, which are composite materials. Gypsum and calcium carbonate layers are produced by surface layer microorganisms. Although bacteria and fungi are used in numerous biotechnological applications, such as the cleanup of hazardous metals, these microorganisms have recently been discovered to be potential eco-friendly nanofactories. A relatively new and mostly unexplored field of study based on the employment of microorganisms in the biosynthesis of nanomaterials has been developed as a result of processes developed by nature for the production of inorganic materials on nano-

and microlength scales. The utilisation of bacteria, actinomycetes, algae, yeast, and fungi in the production of metal nanoppapers and their applications is now the subject of extensive study worldwide.

Nanoppaper Synthesis and Fungi

The list of potentially important microorganisms now includes the use of fungus in the manufacture of nanoppapers. Fungi have the potential to be quite fascinating since they release a lot of enzymes and are easier to handle in the lab. However, eukaryotic species need more genetic engineering than prokaryotes do in order to overexpress certain enzymes found in the creation of nanomaterials. Two genera were identified after a thorough screening procedure, and when exposed to aqueous metal ions like AuCl and Ag+, they produced significant amounts of extracellular or intracellular metal nanoppapers. After being exposed to the 4 M HAuCl4 solution, Verticillium's biomass developed a characteristic purple hue, which denoted the intracellular production of gold nanoppapers. Higher magnification made it possible to observe the 5- to 0-nm-sized nanoppapers with an average size of around 8 nm filling the cytoplasmic membrane and cell wall of the fungal cell. Additionally, the biofilm's powder diffraction pattern demonstrated the crystalline structure of gold nanoppapers. The intracellular development of silver nanoppapers was identical when Verticillium sp. was exposed to silver ions[6]. Now that it has been characterised and documented, microorganisms including bacteria, yeasts, algae, fungus, and actinomycetes may be used in the production of metal nanoppapers. In the multidisciplinary area of "bionanotechnology," engineers, physicists, and chemists must work together. Before such biosynthetic techniques can compete with conventional protocols, a number of challenges need to be resolved from the perspectives of nanotechnology and microbiology. The development of an efficient method for the manufacture of microbial nanoparticules requires the identification of metabolic processes that lead to metal ion reduction among the many groups of microorganisms. It's important to understand the surface chemistry of biogenic nanoppapers as well. Techniques for genetic engineering may be utilised to enhance the characteristics of ppapers and regulate their composition. The use of fungus rather than bacteria to create natural "nanofactories" has the additional benefit of making downstream processing and management of the biomass considerably easier. Currently, only a few metals, a few metal sulphides, and a very small number of oxides can be synthesised using microbial techniques, which is highly limiting. Microbial synthesis may become a viable business idea if the processes were expanded to make it possible to reliably create nanocrystals of additional oxides, nitrides, and carbides.

Various Fresh Applications

Innovative findings in microbial ecology, which offer up new applications and research in microbial technology, are used to highlight the advancement in a number of cases. Here is a quick description of a few of these uses.

Climate Change and Microbes

Scientists, government officials, and the general public are presently very concerned about the manmade creation of carbon dioxide. However, it only amounts to % of the CO2 that soil would ordinarily create. In the range of 0.3 to 1.0 tonnes of carbon per hectare per year, humus may be allowed to build up in the soil via sustainable agriculture. In this approach, high-quality agriculture may be used to offset approximately% of all the carbon emissions from autos. Furthermore, nothing is known about how temperature and pCO2 affect microbial behaviour. The "homeostasis" of the microbial communities may be able to adjust for changes in land use and climate[7].

It is generally known that soil microorganisms play a key role in the production and removal of CO2 and NO2. Comparably, it has been shown that the rumen microbiology is responsible for a small portion of total methane emissions, and fresh research towards short-circuiting rumen methanogens by feeding alternative electron acceptors such herbal components is now underway. Another significant source of methane emissions are rice fields, and one fascinating possibility is to use electrodes to capture the reducing equivalents in the mud layer. Waste dumps rank third in terms of methane emissions, and here contemporary biotechnology's anaerobic digestion technique gives the chance to catch these emissions and utilise them to create usable energy. However, the services provided to reduce methane by the methanotrophic bacteria found in soil are the most important of all. On the order of 0-1,0 kg CH4 per acre per year, these bacteria scavenge. It is obviously necessary to comprehend their function and the breadth of their capabilities. Additionally, one should be able to stop them from being impeded by the use of fertilisers and pesticides and even consider sowing and strengthening them in areas of concern, such the forest phylosphere, which produces methane. The fields of the production of biohydrogen, bioethanol, biodiesel, biogas, and bioelectricity have a variety of innovative views and uses. Even the possibility of gathering solar energy using a combination of legumes and hydrogen-producing Rhizobium symbionts has been theorised. Plans to use biogas technology in conjunction with microbial fuel cell technology to extract sediments, wastes, and downstreams from biorefineries will also continue to spark interest.

Microorganisms and Health

Elie Metchnikoff, a Russian who won the Nobel Prize, was the first to postulate that certain bacteria may alter the makeup of the gut flora. He hypothesised that the lifespan of Steppes Bulgarians and Russians was caused by their use of "sour milk," which in reality is probably lactic acid bacteria like Lactobacillus bulgaricus. Babies with diarrhoea should be given the germs that Henry Tissier of the Pasteur Institute identified from the faeces of healthy breast-fed newborns. Yakult, a probiotic beverage created by Minoru Shirota in Japan in, includes Lactobacillus casei Shirota, a strain that can survive stomach transit and colonise the intestine. The probiotic market is now estimated to be worth around $6,000,000 yearly and is expanding at a rate of about%. Since, more than 2,0 probiotic patent applications have been submitted, with around 4 of them being awarded. The LAB members lactobacilli and bifidobacteria are the two probiotics that are employed in commercial goods the most often. The probiotic potential of certain yeast and other bacteria has also been suggested, however. Siezen and Wilson provide a review of the commercially available strains, their alleged probiotic properties, and their genomes[8], [9].

The most popular form of probiotics is found in yoghurt and beverages that resemble it. Humans that use probiotics are hoping to preserve or enhance their intestine's health. Probiotics are said to work in a variety of ways, including enhancing the function of the intestinal barrier, regulating immunological responses, supplying vitamins, and combating infections. Readers are advised to consult the works of Ventura et al., Kalliomaki et al., Lebeer et al., Kleerebezem and Vaughan, and Siezen and Wilson for current reviews.Probiotic strains must have favourable technical qualities for manufacturing and storage as well as minimal health risks to consumers from an industrial standpoint. Probiotics don't only have to be consumed orally or used in food. Some have been used to treat vaginal infections and may be used topically as lotions or creams. Additionally, probiotics are used as growth promoters in fish and animal feed in place of forbidden additives like antibiotics or growth hormones. They seem to function by inhibiting or lowering the burden of harmful germs that certain fish or animals carry.The GOLD database contains publicly accessible

whole genome sequences of suspected probiotic bacteria, including Bifidobacterium and Lactobacillus species.

CONCLUSION

In conclusion, microbial-based technologies have proven to be effective in the removal of metals from water. These technologies provide a cost-effective, environmentally friendly, and sustainable alternative to traditional physico-chemical methods. The use of microbes in metal removal from water is based on their unique ability to adsorb, accumulate, and transform metals. Microbes such as bacteria, fungi, and algae have been extensively studied for their metal removal capabilities. In recent years, research has focused on optimizing the conditions for microbial metal removal, identifying the mechanisms involved, and developing new microbial strains with enhanced metal removal capabilities. Although microbial-based technologies have shown promise in metal removal, challenges such as scaling up and maintaining the microbial biomass under various environmental conditions remain to be addressed. Nevertheless, the potential of microbial-based technologies in metal removal from water is significant and holds promise for the development of sustainable and effective solutions to address the challenges of metal pollution in aquatic environments.

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CHAPTER 3

CULTURE METHODS IN MICROBIAL ECOLOGY: APPLICATIONS AND LIMITATIONS

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ABSTRACT:

Microbial ecology is the study of microorganisms and their interactions with each other and with their environment. The complexity and diversity of microbial communities have led to the development of various methods for studying microbial ecology. This review provides an overview of the methods commonly used in microbial ecology, including culture-based and culture-independent techniques. Culture-based techniques involve the isolation and cultivation of individual microorganisms, while culture-independent techniques rely on DNA-based methods to characterize microbial communities without the need for cultivation. The review discusses the advantages and limitations of different methods, as well as their applications in studying microbial diversity, community structure, and function. Furthermore, the review highlights recent advances in microbial ecology methods, such as high-throughput sequencing, single-cell genomics, and functional genomics, and their potential to provide new insights into the ecology of microorganisms. The review also discusses the challenges and future prospects of microbial ecology methods, emphasizing the need for interdisciplinary approaches and integration of different techniques to achieve a comprehensive understanding of microbial communities.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Many of the commercial probiotic strains available today come from the intestines of young children and healthy adults. The emphasis of current research is on identifying the traits that these bacteria utilise to thrive and compete successfully in the gut. Using this information, more potent probiotic strains may be found. Numerous gut metagenomic sequencing initiatives are being conducted globally to find possible novel probiotic candidates, which will hasten this search. Additionally, have a look at the Human Gut Microbiome Initiative and the Human Gut Metagenome Initiative.Perhaps in the future, similar to the cocktails now used for immunisation against infectious illnesses, we will be introduced to health-promoting beverages that include a variety of probiotic strains. On pub quiz evenings, memory-boosting beverages may be a financial success, as proposed by Siezen and Wilson.

The pace of resource use and environmental disruption has increased in response to the fast growth in global population. The need to maintain and improve agricultural output and human health, on the other hand, is stronger. The primary bioresource to be used to address the most pressing problems of the twenty-first century is the untapped variety of microbial

life and the readily accessible, culturable microorganisms. Different extreme environments' genetic potential is thought to be advantageous for industrial technologies. Applications of microorganisms for the advancement of environmental quality, agricultural production, human health, and innovative purposes including combating global climate change, nanomaterials, biosensors, biofuels, and probiotics would greatly benefit from future study and extension[1].Research on plant health, plant genome promotion, bioremediation, metagenomics, functional microbial genomics, novel applications in sustainability of the environment, and the role of microbes in global climate change, crops, new drug development, and transgenic development should all be prioritised as areas of focus for our future work.

Molecular Methods to Examine the Dynamics, Function, and Structure of Microbial Communities in the Environment However, since they focus on a single population of microbes, culture-based approaches are incredibly biassed in their assessments of the genetic diversity of the microbes present in natural and anthropogenically altered ecosystems. Microbial community investigations employing culture-independent molecular approaches have started a new age of microbial ecology thanks to recent advancements in genomics and sequencing technology. According to molecular investigations of environmental communities, the cultivable fraction makes up less than 1% of all prokaryotic species found in any given sample. Numerous molecular techniques have been investigated and have provided structural and functional details regarding microbial communities. These techniques are based on direct isolation and analysis of nucleic acids, proteins, and lipids from environmental samples. The huge variety of microbes must be identified and characterised, and their interactions with biotic and abiotic environmental variables must be understood.

Molecular methods like genetic fingerprinting, metagenomics, metaproteomics, metatranscriptomics, and proteogenomics are essential for this. This chapter reviews current developments in molecular microbial ecology with a focus on cutting-edge methods and strategies that provide fresh perspectives on the phylogenetic and functional diversity of microbial assemblages. The benefits and drawbacks of popular molecular techniques for analysing microbial populations are reviewed. Examples studies have been used to demonstrate the possible uses of each molecular approach as well as how they might be combined to provide a more thorough evaluation of microbial diversity[2].

The biosphere is composed of 4-6 prokaryotic cells and is dominated by microbes. This amount is at least two to three orders of magnitude larger than the sum of all animal and plant cells. Microorganisms make up a much diversified category of organisms and account for % of the biomass on Earth. The number of microbial cells in aquatic habitats, such as the seas, has been estimated to be about 1.2, whereas soil in terrestrial environments may support up to 4-5 microbial cells. Microorganisms make up a significant portion of the Earth's biota and constitute a sizable untapped store of genetic variation because of their massive numbers. Microbial ecology has a high priority for understanding this untapped genetic diversity from viewpoints like the greenhouse effect and global climate change.

Important ecological processes include the development of soil structure, the breakdown of organic matter and xenobiotics, and the recycling of vital minerals and nutrients all involve microorganisms as critical participants. Thus, bacteria have an impact on all life on Earth and are crucial in controlling global biogeochemical cycles. In actuality, microbial activities affect every life in the biosphere, either directly or indirectly. Microorganisms have a key role in soil ecosystems in preventing soil-borne plant illnesses, fostering plant development, and encouraging changes in vegetation. Bioremediation methods, energy production methods, and

biotechnological sectors including the pharmaceutical, food, chemical, and mining all need a knowledge of microbial dynamics and their interactions with biotic and abiotic variables.

DISCUSSION

What kind of microorganisms are present? How do they interact with one another? These are the three main concerns that arise when identifying and characterising any natural or artificial ecosystem. What function do these microbes serve? And how are these microbes' actions related to how an ecosystem works? By examining how microorganisms interact with one another and their surroundings, microbial ecology seeks to provide answers to these fundamental issues. Many biochemical and molecular techniques have been used to analyse the composition of microbial communities in response to environmental changes throughout time and place. These innovative methods enable the connection between ecological processes in the environment and particular microbial populations, and they aid in our understanding of crucial issues in microbial ecology, such as the reasons and resources that control the vast genetic and metabolic diversity seen in an environment. This chapter provides an overview of the current molecular techniques utilised in microbial ecology, including their advantages and disadvantages. Although the strategies have been addressed with a focus on soil and plant microbial eco- systems, they may also be used in a wide range of other habitats, including sediments and the ocean.

Applications and Limitations of Microbial Ecology Culture Methods

The isolation and characterisation of microorganisms using commercial growth media, such as Luria-Bertani medium, Nutrient Agar, and Tryptic Soy Agar, are standard culture methods to characterise microbial ecology. The main drawback of culture-based methods is that >% of the organisms seen under a microscope in any environment cannot be grown using conventional culturing methods. To increase the proportion of microbial communities that are cultivable, a number of enhanced cultivation techniques and culture media have been developed that match natural environments in terms of nutrients, oxygen gradient, pH, etc. The encapsulating of cells in gel microdroplets for large-scale microbial growth under low nutrient flow circumstances is one method that has been developed for the cultivation of uncultured microorganisms from various habitats, such as soil and saltwater. These organisms, while viable in their native surroundings, do not thrive under laboratory circumstances and stay in a "viable but nonculturable" state. However, not all "uncultured" species are cultivable, and many of them remain "unculturable." Such VBNC species may represent whole new groupings, may be numerous or very active, yet remain unexplored by conventional culture techniques[3].

S rRNA gene-based molecular microbial surveys show that ancient bacterial divisions including BRC1, OP, OP, SC3, TM7, WS2, and WS3 do not have cultured representatives and are only recognised by their molecular sequences. Due to the absence of any cultural representatives, these division-level clades, such as OP, are regarded as "candidate divisions" to reflect our restricted understanding. They are extraordinarily varied and extensively spread in many habitats. According to studies, there are at least two phyla of bacteria, of which only one is totally represented by molecular sequences. The majority of the time, microorganisms recovered using conventional culture techniques are neither numerically abundant nor functionally relevant in the environment from which they were cultivated. These cultivated bacteria, which make up less than 1% of all microbial species, are regarded as the "weeds" of the microbiome. Due to their ease of cultivation in laboratories, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria make up the majority of the isolates produced from soil samples. Although acidobacteria make up a percentage of soil bacterial populations, only a

few genera are known to include these species. These results imply that for comprehensive characterization of ambient microbial populations, molecular approaches that do not need isolation and culturing are extremely desired.

Analyses of Microbial Communities Using Molecular Techniques

Most microbial communities seen in nature have never been cultivated in a lab. Therefore, these uncultivated yet functional organisms' biomolecules, such as their lipids, proteins, and nucleic acids, are the main sources of knowledge about them. Analysis of whole genomes or specific genes, such as S and S rRNA for prokaryotes and eukaryotes, respectively, are two examples of culture-independent nucleic acid techniques. Cellular life has been divided into three major domains: one eukaryotic and two prokaryotic, based on comparisons of these rRNA signatures. The study of microbial ecology has advanced significantly over the last several decades, and many molecular methods have been created to describe and characterise the phylogenetic and functional diversity of microorganisms. According to their capacity to expose the structure and function of the microbial diversity, these methods have been broadly divided into two categories: partial community analysis approaches and whole community analysis approaches.

Approaches to Partial Community Analysis

Typically, these approaches involve polymerase chain reaction-based techniques, in which total DNA/RNA taken from an environmental sample is used as a template for the characterisation of microorganisms. The PCR result so produced, including the VBNC fraction, displays a combination of microbial gene signatures from all species present in the sample, in theory. Since S rRNA and other conserved genes are present in all prokaryotes, are structurally and functionally conserved, and contain variable and highly conserved regions, PCR amplification of these genes from an environmental sample has been widely used in microbial ecology. Additionally, it is a "gold standard" option in microbial ecology due to the acceptable gene size and increasing amount of S rRNA sequences that are accessible for comparison in sequence databases. The closest phylogenetic affiliation of a novel isolate or molecular sequence is determined by evaluating the phylogenetic relatedness to known microorganisms based on the homology of S rRNA sequences. In microbial research and to distinguish certain bacterial species, other conserved genes such RNA polymerase beta subunit, gyrase beta subunit, recombinase A, and heat shock protein have also been employed. The primary methods used to analyse the PCR products generated from ambient DNA include the clone library method, genetic fingerprinting, DNA microarrays, or a combination of these methods[4].

Library Method for Clone

Cloning and sequencing individual gene fragments is the most used technique for analysing PCR products produced from environmental samples. A database like GenBank, the Ribosomal Database Project, or Greengenes is used to compare the acquired sequences to known sequences. At sequence similarity cut-off values respectively, cloned sequences are often allocated to phylum, class, order, family, subfamily, or species. Studies have demonstrated that environmental samples like soil may take over,0 clones to record% of the richness, despite the fact that clone libraries of S rRNA genes provide an initial scan of diversity and the identification of new species.

However, conventional S rRNA gene clone libraries often include fewer than 1,0 sequences and as a result, only partially disclose the variety of microorganisms present in a sample. The microbial community composition of mining-impacted deep subterranean soils of the former

Homestake gold mine in South Dakota, USA, was deciphered using a cloning-and-sequencing approach. Only a limited picture of the phylogenetic breadth contained in soil samples could be seen from phylogenetic analysis of 0 clone sequences. Due to inadequate clone sequencing, a typical issue when evaluating environmental microbial diversity using cloning techniques, rarefaction analysis of clone libraries produced nonasymptotic plots that showed diversity was not fully examined. Clone libraries are still regarded as the "gold standard" for first studies of microbial diversity despite their drawbacks. Great advancements in this technique of microbial diversity study are anticipated with the introduction of more recent, less costly sequencing techniques[5].

Techniques for Genetic Fingerprinting

A profile of microbial communities is produced by genetic fingerprinting based on a direct examination of PCR products generated from ambient DNA. The community fingerprint generated by these methods, which include DGGE/TTGE, SSCP, RAPD, ARDRA, T-RFLP, LH-PCR, RISA, and RAPD, is based either on sequence polymorphism or length polymorphism. Generally speaking, genetic fingerprinting techniques are quick and enable the analysis of numerous samples at once. In contrast to straightforward taxonomic identification, fingerprinting techniques have been developed to show an impact on microbial communities or distinctions between microbial communities. Software programmes like GelCompar examine the "fingerprints" of several samples using computer-assisted cluster analysis to determine community ties. Community fingerprints are classified as present or missing, and the Jaccards' coefficient is used to assess sample similarity.

Gel Electrophoresis with a Temperature Gradient or Denaturing

When doing denaturing-gradient gel electrophoresis, environmental DNA is used to generate PCR products, which are then electrophoresed on a polyacrylamide gel with a linear gradient of DNA denaturant, such as a solution of urea and formamide. The same principles that underlie DGGE also apply to temperature-gradient gel electrophoresis, with the exception that a temperature gradient is used in place of a chemical denaturant. Amplicons with different sequences cease migrating at various locations in the gel because sequence diversity across various PCR amplicons impacts melting behaviour. In the PCR stage of both DGGE and TTGE, a 5'-GC clamped forward primer is used. This is crucial to avoid the total dissociation of the two DNA strands into single strands during electrophoresis. The bands may be removed from the gel, reamplified, and sequenced to determine the phylogenetic identities from DGGE/TGGE fingerprints, or they can be blotted onto nylon membranes and hybridised to molecular probes unique to certain taxonomic groupings. Using universal bacterial primers from soil microbial communities, DGGE profiles are often highly complicated. Group-specific PCR-DGGE with primers solely targeting certain physiological/phylogenetic groups has been utilised to solve this issue[6].

The following are some more issues with DGGE/TGGE: insufficient DNA sequence data for phylogenetic analysis bands, a number of distinct DNA fragments that can be separated by polyacrylamide gel electrophoresis, and sequence heterogeneity among multiple rRNA operons of one bacterium all contribute to the formation of multiple bands in DGGE, which could lead to an overestimation of diversity. The number of operational taxonomic units and unique clones in clone libraries have been determined using DGGE analysis based on different patterns. DGGE was used on soil samples taken from several agricultural areas in Norway and the USA that were subjected to various agronomic treatments as part of a microbial community analysis. One of these soil samples also had a significant polyaromatic hydrocarbon contamination. Primers based on the V3 and V6/V9 regions of S rRNA for the

bacterial population and the V3 region of S rRNA for archaeal populations were used to create DGGE profiles. Results indicated that, with the exception of the soil sample containing PAHs, bacterial diversity was much higher than archaeal diversity.

Monomorphic Single-Strand Conformation

The environmental PCR products are denatured in single-strand conformation polymorphism, which is followed by the electrophoretic separation of single-stranded DNA fragments on a nondenaturing polyacrylamide gel. Sequence changes that result in a distinct folded secondary structure and a quantifiable differential in mobility in the gel are the basis for separation. SSCP technology is a less complicated and convoluted method than DGGE since it doesn't need gradient gels, GC clamped primers, or other specialised electrophoretic equipment. The DNA bands may be removed from the gel, reamplified, and sequenced, much as with DGGE. However, SSCP works well for tiny pieces only. The high rate of reannealing of DNA strands after initial denaturation during electrophoresis is a significant drawback of the SSCP technique. This issue may be resolved by employing a phosphorylated primer during PCR, followed by selective digestion of the phosphorylated strand using lambda exonuclease. Pure cultures of Bacillus subtilis, Pseudomonas fluorescens, and Sinorhizobium meliloti that were isolated from soil samples were effectively distinguished using SSCP. Additionally, these scientists used SSCP to examine the bacterial communities found in the rhizosphere of two distinct plant species, Medicago sativa and the common weed Chenopodium album. Despite the fact that both plants were grown in the same soil, their findings demonstrated that each plant had its own unique rhizosphere bacterial community.

DNA Amplification and Random Amplified Polymorphic DNA Fingerprinting

Use of PCR amplification with a short primer, which anneals randomly at numerous places on the genomic DNA under low annealing temperature, usually °C, is a key component of random amplified polymorphic DNA and DNA amplification fingerprinting methods. Depending on the genetic complexity of the microbial communities, these approaches produce PCR amplicons of different lengths in a single reaction that are separated on an agarose or polyacrylamide gel. RAPD/DAF has been widely employed in fingerprinting the overall microbial community structure and closely related bacterial species and strains because to its high speed and simplicity of usage. Both RAPD and DAF are very sensitive to the experimental setup, amount and quality of primers, and template DNA. To analyse the relatedness of microbial communities and produce the best discriminating patterns between species or strains, several primers and reaction conditions must be investigated. To measure changes in microbial diversity in soil samples treated with pesticides and chemical fertilisers, a RAPD profiling analysis using random primers was utilised. Pesticide-treated soil retained about the same degree of DNA diversity as the control soil, according to RAPD fragment richness data. Contrarily, as compared to control soil, chemical fertiliser led to a decline in DNA diversity[7].

Ribosomal DNA Amplified Restriction Analysis

Based on DNA sequence variants found in PCR-amplified S rRNA genes, amplified ribosomal DNA restriction analysis is performed. Tetracutter restriction endonucleases are often used to digest the PCR product generated from ambient DNA, and limited fragments are then resolved on agarose or polyacrylamide gels. The ARDRA approach is nonetheless effective for quick monitoring of microbial communities over time or to compare microbial diversity in response to changing environmental circumstances even if it offers little to no information about the kind of microorganisms present in the sample. In environmental clone libraries, ARDRA is also utilised to determine the distinctive clones and estimate OTUs

based on restriction profiles of clones. One of the main drawbacks of ARDRA is that agarose/PAGE resolution of restriction profiles derived from diverse microbial communities is sometimes too challenging. The impact of cop- per pollution on the microbial communities in soil was evaluated using the ARDRA approach. In comparison to control soil with no contamination, ARDRA profiles for the whole community revealed less variety in soil polluted with copper[8].

With one significant exception the use of a single 5 fluorescently labelled primer during the PCR reaction Terminal Restriction Fragment Length Polymorphism and ARDRA are both types of restriction fragment length polymorphism. Following restriction enzyme digestion of the PCR products, terminal restriction fragments are sorted on an automated DNA sequencer. The banding pattern is made simpler by just detecting the terminally fluorescently labelled restriction pieces, enabling the investigation of intricate microbial communities. By examining the size, quantity, and peak heights of the generated T-RFs, community diversity may be inferred. It is hypothesised that each T-RF corresponds to a single OTU or ribotype. Many Web-based T-RFLP analysis programmes have been created as a result of recent advancements in bioinformatics, allowing researchers to quickly assign putative identities based on a database of fragments generated by known S rDNA sequences. Similar to ARDRA, a T-RFLP pattern identifies the used restriction enzyme, and often more than two enzymes are utilised. Because only a small number of bands per gel can be resolved and multiple bacterial species might share the same T-RF length, one drawback of T-RFLP is that it underestimates community diversity. However, the approach does provide a reliable indicator of community variety, and T-RFLP findings often show a strong correlation with those from clone libraries. To comprehend the biogeographical patterns in soil bacterial communities and to look into the biotic and abiotic elements that affect their composition and diversity, Fierer and Jackson used the T-RFLP approach.

CONCLUSION

In conclusion, culture-dependent methods have been widely used in microbial ecology to isolate and identify microorganisms from environmental samples. These methods have several advantages, including the ability to obtain pure cultures for detailed physiological and biochemical studies, and the potential for biotechnological applications. However, culture-dependent methods also have several limitations, such as the inability to culture the majority of microorganisms present in environmental samples, resulting in an incomplete understanding of microbial diversity and function. Additionally, culture-dependent methods may introduce bias towards fast-growing and easily cultured microorganisms, leading to an over-representation of certain taxa and potentially missing key microbial players in environmental processes.

Recent advances in culture-independent methods, such as metagenomics and single-cell genomics, have enabled the identification and characterization of uncultured microorganisms, providing a more comprehensive view of microbial diversity and function. Nevertheless, culture-dependent methods remain important for the isolation and study of microorganisms with unique properties and the development of novel biotechnological applications. In conclusion, culture-dependent methods continue to be valuable in microbial ecology, despite their limitations. A combination of culture-dependent and culture-independent methods is necessary to obtain a comprehensive understanding of microbial diversity, function, and biotechnological potential. Furthermore, ongoing research and development of new culture methods are needed to overcome the limitations of current methods and facilitate the discovery of new microbial taxa and functions.

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CHAPTER 4

ANALYSIS OF MICROBIAL DNA TECHNOLOGY

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ABSTRACT:

Microbial DNA technology has revolutionized the field of microbiology, enabling the identification and characterization of microorganisms from diverse environments, including soil, water, and the human body. This technology involves the isolation, amplification, and analysis of microbial DNA using various molecular techniques, such as polymerase chain reaction (PCR), next-generation sequencing (NGS), and bioinformatics. These techniques have enabled researchers to study microbial diversity, community structure, and function in unprecedented detail, providing new insights into the roles of microorganisms in biogeochemical cycles, human health, and disease. This review provides an overview of the principles and applications of microbial DNA technology, including the advantages and limitations of different molecular techniques. The review also discusses recent advances in microbial DNA technology, such as single-cell genomics, metagenomics, and transcriptomics, and their potential to further advance our understanding of microbial communities and their functions.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

In order to represent a broad variety of temperature, pH, and other geographic factors, they gathered soil samples from all across North and South America. Their findings showed that bacterial diversity was greater in neutral soils than in acidic soils, and that it was independent to elements like site temperature, latitude, and other elements that are generally strong indicators of animal and plant variety. Length heterogeneity PCR analysis is similar to T-RFLP analysis, but LH-PCR distinguishes between different microorganisms based on natural length polymorphisms that result from gene mutation. T-RFLP analyses variations in amplicon length that are produced after restriction digestion. Amplicon LH-PCR examines the hypervariable S rRNA gene regions and generates a distinctive profile. In LH-PCR, the forward primer is fluorescently dye-labeled, and each sample is run with a fluorescent internal size standard to quantify the amplicon lengths in base pairs. The relative abundance of that specific amplicon is proportional to the intensity or area beneath the peak in the electropherogram[1].

When compared to T-RFLP, LH-PCR has the benefit of not requiring restriction digestion, allowing for direct fluorescent detector analysis of the PCR products. Phylogenetically different taxa may generate identical-length amplicons, which is one of the limitations of the LH-PCR technology. Another limitation is that it is unable to resolve complicated amplicon peaks. To examine the microbial communities in soil samples that varied in type and/or crop

management practises, LH-PCR was combined with fatty acid methyl ester analyses.Results from the LH-PCR showed a great correlation with the FAME analysis, were very repeatable, and effectively distinguished between various soil samples. Based on cloned LH-PCR results, members of the -Proteobacteria, Cytophaga Flexibacter Bacteriodes, and the high-G + C-content Gram-positive bacterial groups were the most numerous bacteria in the community.

Analysis of the Ribosomal Intergenic Spacer

The intergenic spacer region located between the small and large ribosomal subunits is amplified using PCR in the process of ribosomal intergenic spacer analysis. There is a lot of length and nucleotide sequence variation in the ISR. Most of the prevalent bacteria found in an environmental sample may provide RISA profiles by utilising primers that anneal to conserved areas in the S and S rRNA genes. With each band belonging to at least one organism in the original community, RISA offers a community-specific profile. The automated RISA procedure, known as ARISA, uses a forward primer that has been fluorescence-labeled, and ISR fragments are automatically recognised by a laser detector. ARISA enables the simultaneous analysis of several samples, although it has been shown that the method overestimates microbial richness and diversity. In order to characterise the bacterial communities from four different kinds of soil with different geographic origins, plant covers, and physicochemical characteristics, Ranjard et al. examined ARISA. These soils produced different ARISA profiles with a number of diagnostic peaks in terms of size and intensity. Their findings showed that ARISA is an extremely useful and accurate technique for identifying variations between complex bacterial populations at different geographical scales[2].

DNA Microarrays

A high-throughput and comprehensive assessment of the microbial communities in environmental samples has mostly been achieved using DNA microarrays. Known molecular probes that are affixed to the microarrays are immediately hybridised with the PCR products that were generated from total environmental DNA. Confocal laser scanning microscopy is used to measure positive signals after the fluorescently labelled PCR amplicons have hybridised to the probes. The ability to quickly examine samples with replication is a key benefit of the microar- ray approach for microbial community investigations. In general, the microarray hybridization signal strength and target organism abundance are directly inversely correlated. A significant drawback of microarray technology is cross hybridization, especially when working with environmental materials. The microarray is also useless for locating and detecting new prokaryotic taxa. If a species' ecological significance is not well represented by a comparable probe on the microarray, it may be entirely overlooked. DNA microarrays for microbial research

About,0 probes of the S rRNA gene are addressed to several cultured microbial species and "candidate divisions" in the global high-density S microarray. These probes can simultaneously identify 8,1 bacterial and archaeal species and target all 1 demarcated prokaryotic orders. Rapid environmental microbial community profiling has been accomplished using PhyloChip technology in bioterrorism monitoring, bioremediation, climatic change, and source tracing of pathogen contamination. In South Dakota's Edgemont and North Cave Hills, two abandoned uranium mine sites, PhyloChips were utilised to look into the local soil bacterial communities. At each taxonomic level, PhyloChip analysis showed more diversity than equivalent clone libraries and demonstrated the existence of 1,0-1,0 bacterial species in soil samples from uranium mines. The majority of these species

belonged to the phylum Proteobacteria and had lineages that could execute metal reduction and uranium immobilization [3].

Arrays of Functional Genes

In contrast to PhyloChips, which are effective in identifying the makeup of microbial communities and which use S rRNA genes as probes, FGA are intended primarily to identify particular metabolic bacterial groups. FGA gave insight on the in situ community metabolic capacity in addition to the community structure. FGA may be used to connect the makeup of microbial communities to the activities of ecosystems since they include probes from genes with established biological functions. One FGA called GeoChip, for example, has over 0 probes from every metabolic gene known to be engaged in a variety of biogeochemical, ecological, and environmental processes including ammonia oxidation, methane oxidation, and nitrogen fixation. The function of Antarctica's soil microbes in the world's biogeochemical cycle of carbon and nitrogen has been examined using GeoChips. Their research showed a strong association between important gene distribution and soil temperature, chemical properties, and plant cover. For instance, microbial carbon-fixation genes were discovered in higher abundance in samples lacking vegetation, and the percentage detection of celluloss degradation genes was connected with temperature[4].

DISCUSSION

Quantitative PCR

In microbial investigations, quantitative PCR, also known as real-time PCR, has been used to quantify the expression and abundance of taxonomic and functional gene markers. In contrast to conventional PCR, which depends on end-point detection of amplified genes, Q-PCR measures the buildup of amplicons in real time throughout each cycle of the PCR using fluorescent probes or intercalating fluorescent dyes like SYBR Green. The measurement of genes when they are proportionate to the initial template concentration is made possible by software that tracks the rise in amplicon concentration during the early exponential phase of amplification. Gene expression may be measured using Q-PCR when it is combined with an earlier reverse transcription process. With a huge dynamic range of almost six orders of magnitude, Q-PCR assesses template abundance while being very sensitive to beginning template concentration. For quick Q-PCR-based quantification of soil bacterial and fungal microbial populations, many sets of S and 5.8S rRNA gene primers have been developed. Targeting the amoA, pmoA, and dsrA genes, respectively, Q-PCR has also been effectively utilised in environmental samples for quantitative identification of significant physiological categories of bacteria, such as ammonia oxidizers, methane oxidizers, and sulphate reducers. By using a Q-PCR test of the pmoA genes, Kolb et al. calculated the abundance of the overall methanotrophic population and particular methanotrophic groups in a flooded rice field soil. Methylosinus and Methylobacter/Methylosarcina groups were the most prevalent methanotrophs, making up 5 to 6 pmoA molecules g1 of the overall population of methanotrophs. The Methylocapsa group was under Q-PCR's detection threshold[5].

Hybridization In Situ With Fluorescence

By whole cell hybridization with oligonucleotide probes, fluorescence in situ hybridization allows for the in situ phylogenetic identification and counting of individual microbial cells. At different taxonomic levels, several molecular probes that target S rRNA genes have been described. The FISH probes are typically - nucleotides long and include a fluorescent dye at the 5' end that enables epifluorescence microscopy to identify the probe attached to cellular rRNA. Additionally, cellular rRNA concentrations and growth rates are associated with

fluorescent signal intensity, which provides information on the metabolic condition of the cells. For a high-resolution automated investigation of mixed microbial populations, FISH and flow cytometry may be used together. In agricultural soils treated with striazine herbicides, the dynamics of bacterial populations were monitored using the FISH technique. Targeting certain phylogenetic groupings of bacteria, such as subgroups of Proteobacteria and Planctomycetes, required the employment of a variety of molecular probes. Results showed that following days of incubation in treated soil compared to control soil with no striazine treatment, populations of -Proteobacteria significantly decreased. Proteobacteria numbers, however, persisted above those of control soils throughout the incubation period. The presence of the herbicide had no discernible effects on other bacterial taxa, such as -Proteobacteria and Planctomycetes[6].

Inaccessible targets, low signal strength, and background fluorescence are frequent issues with FISH analysis. The use of brighter fluorochromes, chloramphenicol treatment to increase the rRNA content of active bacterial cells, hybridization with probes carrying multiple fluorochromes, and signal amplification with reporter enzymes are just a few of the significant advancements that have been made in recent years to address some of these issues. In a modified FISH procedure known as catalysed reporter deposition FISH, the hybridization signal is improved by using fluorochromes that have been tyramide-labeled. As a result, several fluorescent probes might accumulate at the target region, increasing the signal's sensitivity and intensity. Li et al. used secondary-ion mass spectrometry with FISH to create an improved imaging method. S rRNA probes are used in situ hybridization as the technique's basis; however, the probes are labelled with a stable isotope or element that is seldom found in biological material. The microbiological identities of cells that have been labelled with stable isotopes are concurrently ascertained in situ by NanoSIMS imaging after the probe has been hybridised. In order to measure the isotopic composition at the single-cell level, spatial resolution of nm was attained using next-generation SIMS sensors[7].

Analysis of Microbial Lipids

Without depending on culture, microbial community characterization using macromolecules other than nucleic acids, such lipids, has been employed. Signature fatty acids exist in microbial cells that may distinguish the main taxonomic groupings within a community. Fatty acids make up a generally consistent component of the cell biomass. By first extracting the fatty acids by saponification and then derivatizing them, the resulting FAMEs are then subjected to gas chromatography analysis. The fatty acids and their related microbial fingerprints are subsequently identified by multivariate statistical analysis by comparing the developing pattern to a reference FAME data- base. The soil sources polluting surface waters were identified using FAME profiling and multivariate statistical techniques. To create FAME fingerprints for accurate soil categorization, a range of reference soils were gathered from land with varying usage over several seasons. Different soil samples produced FAME fingerprints that could distinguish between reference soils. The findings demonstrated that FAME analysis may correctly categorise sediment samples as long as reference soil FAME profiles are created for samples of surface water and soil taken simultaneously.

Approaches to Whole-Community Analysis

Most microbial ecological surveys employ S rRNA gene sequence analysis. The S rRNA gene, although being a highly conserved molecule, does not provide enough resolution at the species and strain levels. Compared to PCR-based molecular methods that focus solely on one or a small number of genes, whole-genome molecular techniques provide a more thorough perspective of genetic variation. These methods make an effort to examine all the

genetic data included in the complete DNA recovered from a pure culture or environmental sample.

Kinetics of DNA-DNA Hybridization

True genome-wide comparison across species is possible by whole-genome DNA-DNA hybridization. As a suggested criteria for bacterial species demarcation, a value of% DDH was put out. Bacterial species with % or higher genomic DNA similarity often share > % of their S rRNA gene sequence. DDH approaches have been updated for use in whole-microbial community analysis even though they were first designed for pure culture comparisons. The DDH approach involves denatured whole community DNA that has been collected from an environmental sample, followed by an incubation period when they may hybridise or reassociate. The level of genomic complexity in the sample has a direct correlation with the rate of DNA reassociation. The rate of DNA reassociation will decrease if the sample has a significant degree of sequence variability. Under some circumstances, the length of time required for half of the DNA to reassociate correlates with genomic variety and may be used as a measure of diversity. 6,0,0 distinct bacterial genomes per gramme of soil have been hypothesised based on DDH data. This figure might be substantially higher since the study may have missed genomes from uncommon and unrecovered species.Fractionation of Guanine-Plus-Cytosine Content

Guanine-plus-cytosine composition of DNA varies amongst prokaryotic species, although only slightly (3–5%) between phylogenetically similar bacterial taxa. Therefore, density-gradient centrifugation based on G + C contraction may be used to fractionate the whole community DNA. The method produces a fractionated profile of all the community's DNA and shows how DNA abundance varies with G + C content. the whole neighbourhood To more accurately measure the overall community variety, DNA is physically divided into highly pure fractions, each of which represents a distinct G + C content. These fractions may then be examined by other molecular methods, such as DGGE/ARDRA. However, since distinct phylogenetic groups may have the same G + C range, the G + C content fractionation approach only offers a limited degree of phylogenetic resolution. Additionally, it takes a significant quantity of DNA and around 4 days to complete. G + C fractionation has been extensively used in studies of soil microbial communities to see how various treatments or management techniques affect them. To compare the effects of pasture vs forest vegetation on Hawaiian soil micro-biological communities, Nüsslein and Tiedje used G + C fractionation, ARDRA, and S rRNA gene sequence analysis. All three methods showed that plants have a significant role in determining the structure of microbial communities, and that switching from vegetative cover to pasture caused a %difference in the microbial community composition[8].

Whole-Genome Sequencing of Microorganisms

Understanding microbial ecology and function involves investigating microbial systems using whole-genome research, which is a thorough and integrated method. The shotgun cloning method is used to sequence entire microbial genomes. It involves removing DNA from pure cultures, randomly fragmenting the obtained genomic DNA into small fragments of about 2 kb, ligating and cloning the DNA fragments into plasmid vectors, and bidirectional sequencing of the DNA fragments. Using specialised computer programmes like MEGAN, the sequences are aligned and stitched together into whole sequences after they have been retrieved. To anticipate the encoded proteins, the sequences are annotated in open reading frames. The molecular level of microbial operations has been revealed by whole-genome

sequencing, which has potential uses in individual and community ecology, bioenergy production, bioremediation, human and plant health, and a variety of businesses.

The whole genomes of several significant microorganisms, including Pseudomonas syringae DC, Desulfovibrio desulfuricans G, and Methanosaeta thermophila, have been sequenced by a number of institutions and labs, including The Institute of Genome Research, the U.S. Department of Energy's Joint Genome Institute, Lawrence Berkeley National Laboratory, and J. Craig Venter Institute. Desulfovibrio desulfuricans G, a model sulfate-reducing – proteobacterium, has a genome sequence that shows there are metabolic pathways by which it may convert hazardous metals like chromium and uranium to less water-soluble species. These molecular discoveries were very important for the bioremediation of metal-contaminated soils or groundwater using sulfate-reducing bacteria. The time and expense required for whole-microbial-genome sequencing studies have been significantly decreased as a result of recent advancements in short-read sequencing methods like pyrosequencing. For evolutionary research, comparative genomics, and proteomics, the large quantity of data obtained from genome sequencing programmes is published in searchable databases that can be mined using a variety of potent bioinformatic tools accessible at the Integrated Microbial Genomes Web server. For instance, the National Centre for Biotechnology Information's Microbial Genomes Resources is a public database for bacterial genome sequencing studies and currently has one full prokaryotic genome.

CONCLUSION

In conclusion, microbial DNA technology has revolutionized the field of microbiology, enabling the identification, classification, and characterization of microorganisms. Advances in DNA sequencing technologies and bioinformatics have enabled the analysis of complex microbial communities and the identification of novel microorganisms. Furthermore, microbial DNA technology has enabled the study of microbial metabolism, physiology, and evolution, and has facilitated the development of biotechnological applications, such as bioremediation, biocatalysis, and biofuel production.

However, microbial DNA technology has its limitations, such as the potential for bias and errors in DNA sequencing, the inability to distinguish between living and dead microorganisms, and the challenges associated with the analysis of complex microbial communities. Furthermore, ethical considerations must be taken into account when using microbial DNA technology for biotechnological applications, such as the potential for unintended environmental consequences. Microbial DNA technology has had a profound impact on microbiology and biotechnology. Ongoing research and development of new techniques and tools are needed to overcome the limitations of current technologies and facilitate the discovery of new microorganisms and biotechnological applications. Furthermore, the ethical implications of microbial DNA technology must be carefully considered to ensure the responsible use of these powerful tools.

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CHAPTER 5

A FUNDAMENTAL STUDY OF METAGENOMICS METHOD

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ABSTRACT:

Metagenomics is the study of collective microbial genomes that are directly extracted from ambient materials and does not need microbial community understanding or culture. Other names for metagenomics include environmental genomics, community genomics, and microbial ecogenomics. Metagenomics essentially excludes techniques that examine just PCR-amplified copies of chosen genes since they do not reveal genetic variation outside of the amplified genes. Metagenomic approaches are founded on the idea that the whole genetic makeup of microbial communities found in the environment may be sequenced and analysed in a manner similar to that used to sequence the full genome of a pure bacterial culture, as was covered in the section above. A variety of settings, including soil, the phyllosphere, the ocean, and acid mine drainage, have been the subject of Metagenomic studies, which have given researchers access to the phylogenetic and functional diversity of uncultured microorganisms.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

In order to comprehend the metabolic functions of uncultured microbes and their interactions with other biotic and abiotic components, metagenomics is thus essential. Environmental Metagenomic libraries have shown to be excellent sources for novel microbial antibiotics and enzymes with potential uses in biotechnology. Following the isolation of whole DNA from an environmental sample, shotgun cloning of random DNA segments into an appropriate vector, transformation of the clones into a host bacterium, and screening for positive clones are the stages involved in building a Metagenomic library. The metagenome of an environment is better covered by Metagenomic libraries with short DNA fragments in the range of 2-3 kb than by those with bigger fragments. According to estimates, at least genomic clones would be needed to recover the genomes from uncommon members of microbial communities. Small-insert DNA libraries may also be used to rebuild metagenomes for genotypic research and to test for traits that are encoded by specific genes. Investigating multigene biochemical pathways requires large-fragment metagenomic libraries. Either functional screening of expressed phenotypes or sequence-driven Metagenomic analysis, which includes large high-throughput sequencing, may be used to screen genomic libraries. Sequence-driven huge whole-genome Metagenomic sequencing reveals several significant genomic properties, including genomic organisations, traits gained from differently related species via horizontal gene transfers, and redundancy of functions in a community[1].

Libraries are screened in function-driven Metagenomic analysis depending on the expression of a chosen phenotype on a particular medium. Environmental Metagenomic libraries have uncovered a broad range of biochemical activity. In soil Metagenomic libraries, for instance,

new antibiotics, microbial enzymes, and proteins have been found. The effective expression of a target gene in a heterologous host, such as E. coli, is a prerequisite for function-driven Metagenomic techniques. As a result, the majority of environmental genes in E. coli express themselves at extremely low levels or not at all. By incorporating Metagenomic DNA into other surrogate hosts including Streptomyces, Bacillus, Pseudomonas, and Agrobacterium, it is sometimes possible to increase gene expression. Highly desired methods are those that can improve the heterologous expression of unidentified genes in host cells. For instance, cloning vectors with strong promoters that can offer extra transcription factors or genetically modified E. coli that can support the translation and transcription of a broad variety of genes are both extremely desired. The frequency of active gene clones producing a trait in a Metagenomic library is often quite low. For instance, just one out of every zero clones in a soil-based environmental Metagenomic library exhibited lipolytic activity. A unique lipolytic clone's DNA and deduced protein sequence showed only a modest degree of identity with well-known lipases, suggesting that it could come from an uncultured organism. Improved high-throughput screening and detection methods are required because of the low frequency of actively expressing clones in Metagenomic libraries.

Techniques for Next-Generation DNA Sequencing Microbial Ecology

Large-scale sequencing technologies are essential in providing an unbiased understanding of the phylogenetic makeup and functional diversity of environmental microbial communities because they enable us to examine ever-deeper strata of the microbial communities. Numerous applications, including whole-genome sequencing, metagenomics, metatranscriptional biology, and proteogenomics, benefit from the capabilities of large-scale sequencing technologies to produce billions of reads at cheap cost and fast speed. Microbial ecology and genomics have undergone a radical transformation as a result of recent advances in novel sequencing chemistries, bioinformatics, and tools. Compared to classic Sanger's dideoxy sequencing of cloned amplicons, next-generation sequencing technologies like Roche/4, Illumina/Solexa, Life/APG, and HeliScope/Helicos BioSciences are significantly quicker and less costly. Several hundred S rRNA gene clones are typically sequenced using the Sanger method, but 4Life Sciences has commercialised a technique called 4 pyrosequencing that enables massive parallel high-throughput sequencing of hypervariable regions of S rRNA genes and provides two to three orders of magnitude more coverage of microbial diversity. The highly variable areas targeted are readily covered in the short read lengths produced by pyrosequencing methods and are small enough to offer enough phylogenetic information[2].

Multiple environmental samples may be mixed in a single run when employing the pyrosequencing approach, and the reads can then be sorted using the nucleotide barcodes that were added to the tem- plates during PCR. The most recent version of the fourth-generation platform 4 Genome Sequencer XLR can produce read lengths greater than 0 bp and around 0 million high-quality bases per -h instrument run with an accuracy of.%. In the next year, third-generation sequencing platforms created by Helicos and Pacific Biosciences are anticipated to be made available. Huge genetic variety may be found in environmental samples like soil, which includes microorganisms from the Eukarya, Bacteria, and Archaea domains. GenBank, the biggest database of microbial sequences, for instance, returns >6,6 sequence entries when the term "soil" is entered.

The abundance of genetic data that is now accessible in databases is proof that nucleic-acid sequencing and genomics have advanced. Up until recently, the majority of molecular microbial surveys used first-generation automated Sanger sequencing. The expense and time required were the primary limiting factors in the Sanger approach, which led to the majority

of research including the sequencing of just a small number of clones. Only the prominent elements of microbial communities that conceal the identification of low-abundance microorganisms are captured by sequencing a small number of clones. Almost every environmental sample, including soil, has a highly diversified "rare biosphere" made up of these low-abundance microorganisms. A potentially endless genetic pool exists in the uncommon biosphere microbial communities, which are mainly undiscovered and could only be discovered by applying next-generation sequencing technology. In a molecular study, the V1 and V2 hypervariable regions of the S rRNA genes were targeted using a large-scale bar-coded pyrosequencing approach to examine geographical variations in soil bacterial populations. In all, 2,9 high-quality sequences, with an average of 1,1 sequences per sample, were produced from the collection of 88 soil samples from North and South America, covering a variety of ecosystems. With an average of at least 1,000 species per soil sample, the results revealed that the microbial communities in soil exhibit immense phylogenetic diversity. Acidobacteria, Alphaproteobacteria, Actinobacteria, Bacteroidetes, and Beta/ Gammaproteobacteria were the most prevalent phyla in all soil samples. The Lauber et al. research showed that the whole range of species diversity was not covered even after sequencing more than 1.5 billion S rRNA gene amplicons. Further proof that soil bacterial communities are very varied and include a sizable "rare biosphere" represented by a huge number of rare, low-abundance species was supplied by this. These research emphasise the value of using large-scale sequencing methods to study the very varied soil microbial populations[3].

DISCUSSION

Linking Community Structure and Function: Functional Microbial Ecology

A major objective of microbial ecology is to comprehend how microbial populations behave in their natural habitats. When determining whether microbial communities are active vs dormant, RNA isolated from environmental samples is more useful than DNA. This is because rRNA and mRNA are regarded as markers of microbial populations that are functionally active. The quantity of rRNA in a cell generally corresponds with the growth activity of bacteria, and the presence of functional genes on mRNA enables the identification of bacteria that are really expressing important enzyme activities in a given environment. In order to gain insights into important microbial processes, a number of genes, including amoA, nifH, nirK and nirS, and dsrA, have been amplified from DNA/RNA isolated from microbial communities. Enzyme-coding genes involved in the utilisation of certain carbon substrates, such as chitin, cellulose, and lipids, might potentially be used to study the variety of microbial catabolism. By amplifying lipase genes using PCR, the variety of lipase-producing bacteria in glacial soil was examined. Sequence analysis revealed the presence of a number of unique lipase-producing species in the soil. The next sections address more sophisticated techniques using stable isotopes that provide more in-depth information on the metabolic processes of microbial communities. These techniques include stable isotope probing, microautoradiography-FISH, and Raman-FISH[4].

Offering a stable isotope-labeled substrate to microbial communities whose utilisation is of interest to understand a crucial biogeochemical process is known as stable isotope probing (SIP). The isotopes are incorporated into the biomass of active microbial communities throughout the growth process using the labelled substrate. The labelled biomolecules are subsequently separated from the biomass using various biochemical procedures, and the molecular approaches are used to determine the phylogenetic identity of the bacteria metabolising the substrate. DNA that can be labelled with C and separated from C by CsCl equilibrium density-gradient centrifugation is used in SIP that relies on DNA biomarkers.

The identification of microorganisms might be achieved by using genetic fingerprinting or clone library approaches to examine the C-labeled DNA. SIP was used to analyse how soil microbial populations were affected by the pesticide 2,4-dichlorophenoxyacetic acid. After being treated with 2,4-D that has been C-labeled, soil samples were incubated for days. Following incubation, labelled DNA from soil samples was purified and utilised to build S rRNA clone libraries. The absorption and breakdown of the herbicide were attributed to bacteria from the genus -Proteobacteria, including Comamonadaceae and Ramlibacter, according to phylogenetic analyses of clone sequences.

SIP has been used with other methods, like FISH and Raman microscopy, to examine the taxonomic identities and activity of microbial communities at single-cell resolution in recent years because to advancements in imaging and spectroscopic techniques. The Raman-FISH technique involves incubating environmental samples with a substrate that has been labelled with a C stable isotope. After incorporation, Raman microscopy, which quantifies the laser light scattered by chemical bonds of several cell biomarkers, is used to construct the spectral profiles of uncultured microbial cells at single-cell resolution. The quantity of light dispersed is influenced by the percentage of stable isotope incorporation in cells, which causes quantifiable peak shifts for labelled cellular components. The Raman-FISH technique has several advantages over traditional SIP/MAR-FISH methods, including substantially greater resolution. Huang et al. investigated groundwater Pseudomonas communities that degrade naphthalene using the Raman-FISH technique. Their findings revealed that different Pseudomonas species and even individuals within the same species varied in their capacity for naphthalene breakdown. This was based on variations in the C content of the various pseudomonad cells[5].

Microautoradiography

The foundation of microautoradiography is the ability to see metabolically active cells using radiolabeled substrate when they are exposed to radiation-sensitive silver halide emulsion. Cells put on a microscope slide are covered with the emulsion. After exposure, excited silver ions form black metallic silver ppapers that may be seen by transmission electron microscopy within or close to the cells. Glucose, acetate, and amino acids are often utilised radiolabeled substrates that provide an overview of the entire metabolic variety. Important physiological processes have been identified in situ using more specialised substrates and selected growth settings. To detect the iron- and sulfate-reducing microbial populations, respectively, radiolabeled iron or sulphate may be used in controlled anaerobic environments. The simultaneous phylogenetic identification of active cells that consume the radioactive substrate is made possible by the use of MAR in conjunction with FISH.

Other techniques, including STAR-FISH, have been created as a result of a modest modification of MAR-FISH. However, STAR-FISH only varies from MAR-FISH in minor technical aspects; the technique's fundamental idea is the same in both cases. By using an internal standard of bacteria with known particular radioactivity and an enhanced fixation process, Nielsen et al. created a quantitative MAR-FISH method that can identify even single cells. The autotrophic nitri-fying bacteria in biofilms were studied using the MAR-FISH method. Heterotrophic bacteria's uptake MAR-FISH was used to directly observe the distribution of C-labeled products produced by nitrifying bacteria. The findings showed that Chloroflexi and Cytophaga- Flavobacterium members play a crucial role in scavenging the dead biomass and nitrifying bacteria's metabolic products, thereby reducing the buildup of organic waste products in the biofilms[6].

Atomic Array

High-throughput functional and phylogenetic screening of active microbial communities is made possible by isotope arrays. The method combines SIP for tracking the profiles of substrate uptake with microarray technology for identifying the taxonomic makeup of active microbial populations. In theory, samples are incubated with a C-labeled substrate, which is absorbed into microbial biomass as it grows. Fluorochromes are used to mark the C-labeled rRNA after it has been isolated from the unlabeled rRNA. A phylogenetic microarray is hybridised with fluorescently labelled rRNA before radioactive and fluorescent signals are detected. The method enables simultaneous investigation of the makeup of microbial communities and the particular substrates used by metabolically active bacteria in diverse microbial communities. The technique's main advantages are that there is no amplification step involved, therefore it is devoid of the biases that come with PCR. Obtaining high-quality rRNA and identifying low abundance yet active microbial communities from environmental samples are two challenges with the technique. The phylogenetic diversity and CO2 fixation ability of ammonia-oxidizing bacteria in nitrifying activated sludge samples were effectively shown by Adamczyk et al. using this approach. According to their findings, the Nitrosomonas lineage dominated the AOB communities in the sludge samples.

Postgenomic Methods

Metagenomics, one of the most current DNA-based molecular approaches, has provided fresh insights into the evolutionary and functional variety of microbiological communities. The limits of DNA-based molecular techniques, however, have become apparent in the postgenomic age. For instance, gene expression information as it happens in situ is not available using DNA-based approaches. Postgenomic techniques like Metaproteomics and Metatranscriptomics can now be used to apply comprehensive Metagenomic databases, which also contain genomic sequences from uncultured microorganisms, to reveal the relationship between genetic potential and functionality in microbial communities. These methods are thoroughly covered in the sections that follow, along with examples of how they may be used to look into the functioning of microbial communities.

Metaproteomics

The broad study of proteins expressed by ambient microbial communities at a certain time is known as Metaproteomics, sometimes known as environmental proteomics. Protein biomarkers are more trustworthy and provide a better picture of metabolic processes than functional genes or even the matching mRNA transcripts of microbial communities, which are other cell components like lipids and nucleic acids. Despite the fact that techniques like SIP/MAR-FISH have been developed for structure-function assessments of microbial communities, these techniques only provide information on the microbial communities linked to a particular biogeochemical process and do not provide a comprehensive picture of the functions of microorganisms. In contrast to these approaches, proteomics provides a complete strategy to qualitatively and quantitatively explore the physiology of microbial communities. In contrast to DNA/RNA molecular approaches like Metatranscriptomics and metagenomics, proteomic profiling of microbial communities may give important information on protein abundances and protein-protein interactions. A changed proteofingerprint, which indicates changes in the functional state of the communities, may be used to determine the physiological reactions of microbial communities to a stress scenario. Once the proteins have been identified, they may be connected to the matching Metagenomic sequences to connect the functions of the metabolic to specific microbial species[7].

In terms of methodology, Metaproteome analysis entails complete protein extraction from an ambient sample. A considerable quantity of protein comes from other creatures, including

protozoa, fungus, and multicellular animals, which further complicates the taxonomic classification of proteins even though in situ protein lysis procedures provide a thorough recovery. As a result, in certain circumstances, microbial cells are first ultracentrifuged away from the ambient matrix before being lysed, allowing for the production of bacterial proteins of considerably greater quality and quantity. To create a community proteofingerprint, the total protein is separated by one-dimensional and two-dimensional electrophoresis. Protein spots are digested after separation and then recognised using a number of strong analytical techniques. Currently, the advancement of chromatographic and mass spectroscopy methods allows for high-throughput proteome analysis of microbial populations.

A very accurate and speedy identification of proteins is possible because to the integration of high-efficiency mass spectrometry with liquid chromatography. A full set of tools that are essential for the identification and characterisation of protein mass fingerprinting data are made available via Web-based services like ExPASy. To find the proteins involved in the biodegradation of chloro-phenoxy acid in soil samples, a metaproteomic technique was used. By incubating soil samples with 2,4-D for a few days, it was first possible to enrich them for bacteria that break down chlorophenoxy acid. After incubation, protein extracts from the soil were extracted, separated by SDS-PAGE, and the identification of protein bands was done using liquid chromatography coupled with mass spectrometry. Membrane transport proteins, molecular chaperones, and 2,4-dichlorophenoxyacetate dioxygenase were all discovered using proteomic research.

Proteogenomics

In metaproteomics, protein sequences could only be recognised with certainty if they had a substantial similarity to proteins that were already included in the databases that were accessible. However, the majority of ambient proteome investigations show only sporadic relationships between proteins and known database sequences. Therefore, it seems likely that most small protein sequences received from metaproteomes would remain unidentified and unable to be attributed to their functional and phylogenetic characteristics. The term "proteogenomics" refers to the combination of the metaproteomic and metagenomic techniques, which has allowed these limitations to be addressed. In community proteogenomics, complete DNA and proteins are isolated from the same sample, allowing for a more certain connection between biological activities and phylogenetic identity. By increasing the identification of protein sequences by meta-genomic analysis of the same sample from which the proteins were extracted, the metagenomic component of the proteogenomic technique plays a very important role[8].

In a research by Delmotte et al., the proteogenomics technique was used to understand phyllosphere bacterial communities. Proteins were recovered from bacterial biomass that was taken from the leaf surfaces of Arabidopsis, clover, and soybean. Tryptic digestion, liquid chromatography-based fragment separation, and mass spectrometric analysis came next. As a result, 2,3 distinct proteins were found among around 500,000 spectra.

The metagenomic data generated from the DNA extracted from the same pool of bacterial biomass significantly increased the number of proteins that could be identified, showing that most of the bacterial communities found in the phyllosphere were genetically distinct from those found in databases.

The three bacterial genera Methylobacterium, Sphingomonas, and Pseudomonas were responsible for the majority of the discovered proteins in the phyllo-sphere proteome. Methylobacterium species that can utilise methanol as a source of carbon and energy were given credit for a large number of proteins involved in methanol oxidation.

Metatranscriptomics

By examining the worldwide transcription of genes by chance sequencing of mRNA transcripts gathered from microbial communities at a certain time and location, metatranscriptomics enables the monitoring of microbial gene expression patterns in natural settings. The measurement of changes in gene expression and their control in response to changing environmental circumstances is especially well suited to metatranscriptomics. The main difficulty in metatranscriptomics is that prokaryotic mRNA transcripts are not polyA tailed, making it difficult to produce complementary DNA. This causes the whole RNA pool to coextract more abundant rRNA molecules, which might result in an excessive amount of background sequences in a large-scale sequencing study[9].

For the gene transcript analysis of marine and freshwater bacterioplankton communities, a method for selectively enriching mRNA by subtractive hybridization of rRNA has been developed and evaluated. This revealed the presence of many transcripts that were linked to biogeochemical processes like sulphur oxidation, assimilation of C1 compounds, and acquisition of nitrogen via polyamine degradation. Since a community's total RNA pool is inherently rich in both functionally and taxonomically relevant components, such as mRNA and rRNA, a "double-RNA" approach has recently been developed to analyse it. This gives researchers a way to look at microorganisms' structural and metabolic activities in a single experiment. Their research used extensive pyrosequencing and transcriptome profiling to generate 3,9 rRNA tags and,3 mRNA tags from nutrient-poor, pH-neutral samples of sandy soil. The data on the phylogenetic makeup of soil microbial communities supplied by the rRNA tags revealed that Actinobacteria and Proteobacteria were the most prevalent, whilst Crenarchaeota were less prevalent in soil samples. The in situ expression of numerous important metabolic enzymes, including ammonia monooxygenase and nitrite reductase, which were involved in ammonia oxidation, could be shown thanks to the mRNA tags. Microbial gene transcripts for the enzymes 4-hydroxybutyryl-CoA dehydratase and methyl-malonyl-CoA mutase, which are involved in Crenarchaeaota's CO2 fixation pathways, were also found.

CONCLUSION

In conclusion, metagenomics has transformed the field of microbial ecology by enabling the study of microbial communities in their natural environments without the need for cultivation. This technology has the potential to reveal the true diversity of microorganisms and their functional capabilities, leading to new discoveries in the areas of biotechnology, bioremediation, and human health. Metagenomics has enabled the identification and characterization of novel microorganisms, metabolic pathways, and functional genes, as well as the reconstruction of microbial community interactions and dynamics. Despite its many advantages, metagenomics has some limitations, such as the need for high-quality DNA sequencing and the computational resources required for data analysis.

Furthermore, the interpretation of metagenomic data can be challenging due to the complex nature of microbial communities, and the potential for false-positive or false-negative results. Metagenomics has opened up new avenues of research and discovery in microbial ecology, and its potential for biotechnological applications is vast.

Ongoing advancements in DNA sequencing and bioinformatics are necessary to overcome the challenges associated with metagenomics and to enable a more comprehensive understanding of microbial communities. Furthermore, interdisciplinary collaborations are necessary to fully realize the potential of metagenomics for the benefit of human health and the environment.

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CHAPTER 6

BIAS IN MOLECULAR COMMUNITY ANALYSIS METHODS

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ABSTRACT:

Similar to culture approaches, molecular techniques have their own drawbacks and are inextricably linked to prejudice. Biases related to DNA extraction, such as incomplete or preferred lysis of certain microbial cells, may alter the diversity and organisation of the microbial population. To reduce any danger of bias, Feinstein et al. recommended using a variety of verified DNA extraction techniques and pooled DNA extracts in PCR-based molecular assays. Inhibition by substances like humic acids, which are often coextracted with DNA isolated from soil, is one potential source of biases related with PCR. Numerous methods for purifying DNA have been developed, however they all result in DNA loss throughout the process, which also affects the results of PCR that follows. Although dialysis or dilution of DNA templates may be used, it affects PCR effectiveness. The quantitative evaluation of microbial diversity is occasionally impacted by the preferred amplification of certain templates due to hybridization efficiency and primer specificity. Additionally, the development of PCR artefacts may provide false findings.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

The discipline of molecular genomics is seeing unheard-of developments as a result of the creation and use of these technologies. We may examine the structural and functional variety of environmental microbial communities using postgenomic molecular techniques, and these analyses show that we have just begun to scratch the surface of the genetic and metabolic diversity found in the Prokaryotes, the most prevalent creatures on Earth. Unanswered are a number of significant issues, including "How many microbial species are there on the Earth?" "What is the extent of metabolic diversity in natural microbial communities?" and "How microbial communities are governed by biological, chemical, and physical factors?" Since the majority of the found genes lack homologous counterparts in data- bases, understanding the functional functions of uncultured organisms still presents a challenging issue. Although significant progress has been made in the application of Metagenomic, Metatranscriptomic, and proteogenomic approaches to characterise microbial communities, many technical challenges still exist, including the extraction of DNA, RNA, and protein from environmental samples, mRNA instability, and low abundance of specific gene transcripts in total RNA[1].

Numerous technical advancements tailored specifically for environmental samples are anticipated in the next-generation sequencing methods, which are currently under development. The evaluation of the enormous quantity of data produced by whole-genome analysis, Metagenomic, and metatranscriptomics techniques also requires the development of

bioinformatics tools. Due to severe biases associated with nucleic acid isolation and PCR, quantitative evaluation of microbial communities is the most difficult task and calls for more sophisticated DNA/RNA extraction techniques for environmental materials. There are benefits and drawbacks to each of the molecular methods that may be used to analyse community structure and function, and none of them gives full access to the genetic and functional variety of complex microbial communities. To examine the diversity, purpose, and ecology of microorganisms, a variety of methodologies should be used. Culture-based and culture-independent molecular approaches should be seen as complimentary rather than antagonistic or exclusive. To provide fresh perspectives on environmental microbiology, an interdisciplinary systems approach incorporating various "omics" technologies to uncover the relationships between genes, proteins, and environmental variables will be necessary. In the next years, multi- "omics" approach development will be a top focus for study.

The great majority of microorganisms in the aquatic environment prefer to exist as connected colonies known as biofilms rather than as free-floating forms, according to scientific research conducted over the last several decades. In general, biofilm growth on surfaces is harmful to both human health and man-made structures; biofilm-related issues include contaminated drinking water, energy loss in industrial systems, and accelerated corrosion in ship hulls and offshore structures. Additionally, biofilms have a number of advantageous functions, including the transformation of nutrients in the rhizosphere of plants and the accelerated biodegradation of organic carbon and other pollutants during wastewater treatment and soil bioremediation. Biofilms have recently shown considerable promise in selective, affordable catalysis and energy conversion processes for the generation of biofuels and microbially powered batteries. The biofilm structure offers the bacteria inside it a number of benefits, such as viscoelasticity, resistance to biocides and antibiotics, and resilience to fluid-dynamic shear stress[2].

Multiple species congregating in biofilm microcosms expands the spectrum of substrates that may be biodegraded and provides excellent versatility for a variety of biotechnological applications. Researchers have developed several strategies to limit biofilm formation in recent years and have shown the connection between biofilm structure and activity. However, the area of using biofilms to degrade contaminants is still in its infancy. Additionally, academic research is still essentially the only source of the techniques that use biofilms for energy conversion, environmental sensing, and "white biotechnology". We want to highlight the most significant and current developments in the area of biofilm-based technologies and their prospective applications in this chapter.

Most microbiologists have hitherto concentrated on free-floating microorganisms cultured on specific medium. The study of microbial disease and physiology has benefited from this form of culturing, yet bacteria are seldom seen in nature as pure cultured planktonic growth. Direct study of a broad range of natural settings has shown that most bacteria are found clinging to surfaces in well-organized communities rather than as free-floating creatures. The communities that are connected to these are called biofilms.

Both therapeutically and industrially, biofilms are crucial. Clinically speaking, biofilms are the cause of a range of illnesses and disorders, including nosocomial infections, dental caries, and other chronic infections. In the public drinking water and energy sectors, biofilms are a major problem. The growth of biofilms impairs reverse osmosis membrane performance, decreases heat transfer efficiency of heat exchangers, increases fuel consumption of ships, boosts corrosion and obstruction of water distribution pipelines, and contaminates food processing equipment. Bio-films, however, have a number of advantages in a number of

different processes, such as biocontrol of plant diseases, corrosion inhibition, waste-water treatment, bioremediation, productive bio-catalysis, and microbial fuel cells[3].

Microbial cells experience considerable alterations in gene expression during the creation of biofilms. Compared to planktonic cells, biofilm cells have a different general metabolism as a result of alterations in gene expression. In addition to improved production of extracellular polymeric substances, typical three-dimensional biofilm architecture, antimicrobial resistance, quo-rum sensing, gene transfer, and external electron transfer, these changes give biofilms a number of special properties that are either absent or poorly expressed in planktonic cells. The biofilm mode of life shields the microbial population against osmotic stress, dehydration, and nutritional shortages. Biofilm permits different physical, chemical, and molecular interactions among its resident microbial population, allowing for the best use of the resources that are at hand. One or more of the aforementioned biofilm features are exploited or determined by the majority of biofilm-based technologies.

For many years, researchers have discussed the potential of biofilms as corrosion inhibitors and biocontrol agents. There aren't many biofilm-based biocontrol agents on the market; further research is required to promote wider use of this technology. Additionally, the majority of biofilm-based corrosion inhibitors have only been studied in the lab. Biofilms have lately been mentioned in relation to energy conversion and biocatalysis processes. Microbial fuel cells are machines that use electrochemical processes involving biofilms that are either present in both the anode and cathode compartments or in just one to convert chemical energy into electrical energy. Ideally, organic materials like carbohydrates, cellulose, and fatty acids are totally oxidised by biofilms in MFCs[4].

MFCs have shown potential in the creation of biosensors, energy recovery from municipal and industrial wastewater, and bioremediation. Only a few pilot-scale uses of this technique are known, despite significant advancement having been reported in laboratory settings. Technologies based on biofilms have shown tremendous promise in the biocatalysis, biotransformation, and bioremediation of heavy metal, hydrocarbon, and xenobiotic contaminants. Biofilms are generated on support medium in biofilm reactors either by exploiting the native microbial populations or by inoculating a desired culture into the reactor vessel. To carry out the intended reaction, the biofilm that has developed on the support medium functions as a self-regenerating catalyst. The increased concentration of active biomass and resilience to short-term toxic shock, which are typical problems in water treatment, are the key benefits of biofilm treatment over traditional methods utilising free-floating microorganisms.

Recent interest in eco-friendly and affordable technology has given biofilm research a boost. Since they can self-heal and endure extreme environmental conditions, microorganisms in biofilms are essentially perfect catalysts. Even while methods based on enzymes may reach higher conversion rates than those seen when employing living organisms, enzymes are prone to a quick loss of activity when used in actual processes. These issues raise prices and decrease environmental sustainability. The goal of current research is to close the knowledge gap between laboratory studies and practical applications. It is anticipated that large companies will become interested in biofilm-related technologies as a result of the more strict economic and energy requirements in the years to come[5].

According to the definition of a biofilm, it is "a microbially-derived sessile community characterised by cells that attach to a substratum or interface or to each other, with the help of gelati- nous extracellular polymeric substances." The "biofilm matrix" is a particular kind of gelatinous extracellular adhesive. The biofilm matrix offers defence against changes in the

environment, biocides, and antibiotics. Furthermore, by trapping and concentrating vital elements like carbon, nitrogen, and phosphorus, it creates a nutrient-rich "microniche" for bacterial cells within the biofilm.

Biofilm Formation Mechanism

Dissolved organic matter known as conditioning film begins to adsorb onto surfaces as soon as surfaces are exposed to an aqueous environment. Glycoproteins, humic substance, proteins, lipids, nucleic acids, polysaccharides, and aromatic amino acids make up the majority of the ingredients in conditioning film. Bacterial adhesion is impacted by the production of conditioning layer because it alters the surface's general physicochemical characteristics, such as surface charge, wettability, hydrophobicity, and surface roughness. Physical attraction factors including Van der Waals forces and electrostatic forces initially hold bacterial cells loosely to the conditioned surface. However, bacterial cells start to create more solid connections after a few hours of contact with the surface. The subsequent production of extracellular adhesive materials results in the bacterial cells adhering to the surface firmly. The cells begin to proliferate after they are firmly adhered to the surface. Following this expansion, the EPS that holds the growing cells together and aids in the development of a typical mature biofilm is overproduced. Cells immersed in the self-secreted polymeric matrix, voids or water channels, and a distinctive three-dimensional structure are all characteristics of the mature biofilm. These spaces or water channels let nutrients and water go deeper into the mature biofilm. Bacterial cells move from the biofilm phase to the liquid phase via a process known as biofilm detachment in order to colonise new surfaces and prevent population density-mediated starvation. Continuous biofilm detachment keeps biofilm development in check and allows it to attain a quasi-steady state[6].

Influence of Biofilm-Based Technologies on Biofilm Properties

Based on the needed features, a particular biofilm is chosen for the development of biofilm-based technologies; some of these properties are covered below.Performance of the biofilm reactor is impacted by extracellular polymeric substances.Through the synthesis of EPS, bacterial cells permanently cling to surfaces or to one another. Water, polysaccharides, proteins, humic materials, nucleic acids, and lipids make up EPS. EPS are crucial for the formation and structure of biofilms, as well as for the use of biofilm in industrial settings.

The buildup of inactive EPS in bioreactors affects the volumetric productivity of catalytic biofilm processes and limits the area available for active cells. The biotransformation activity of the Acetobacter xylinum biofilm was shown to have decreased by six times as a result of the high EPS concentration, according to a recent study by Setyawatia et al. Upstream processing and product purification are hampered by excessive EPS sloughing. Therefore, EPS synthesis and buildup during industrial biofilm application must be carefully monitored and managed.

Performance of Biofilm Reactors: The Role of Biofilm Architecture

The architecture of biofilms is varied in both space and time and is dynamic owing to both internal and external forces. Since they influence biomass holdup and mass transfer, biofilm architectural traits including thickness, density, and surface shape are essential for the stability and effectiveness of a biofilm reactor. For instance, whereas thick biofilms provide an outside aerobic zone and an interior anaerobic zone, thin biofilms allow oxygen to seep into the deepest levels. Controlling biofilm thickness is crucial for the proper functioning of biofilm reactors. Since fluffy biofilms and outgrowth cause biofilm instability, biofilm surface form is also important [7].

DISCUSSION

Using Quorum Sensing to Clean Out Bioreactors

Bacteria create acylated homo-serine lactones and oligopeptides, among other diffuse extracellular signalling molecules, to keep track of their population density and to coordinate the expression of certain sets of genes in response to it. "Quorum sensing" is the name given to this form of cell density-dependent gene regulation. The first work demonstrating the function of quorum sensing in the development of biofilms was published by Davies et al., sparking a period of investigation into cell-to-cell signalling in biofilms. Every stage of biofilm growth is impacted by quorum sensing, including the dispersion stage, which enables cells to break out from biofilms and colonise new niches. Quorum sensing and biofilm dispersion in industrial biofilm processes are presently poorly understood. In order to increase productivity and make bioreactor cleaning easier, it may be possible to regulate biofilm formation in industrial applications once these processes are well understood[8].

The Importance of Antimicrobial Resistance in Bioreactor Cleanup

Compared to suspended cells, biofilms are widely recognised for having a higher tolerance for poisonous substances, antimicrobials, and other unfavourable circumstances. Reduced diffusion within biofilms, constrained cell growth, the development of specialised survivor cells known as persisters, and active toxin clearance are a few resistance mechanisms that contribute to this trait. Some of these biofilm defence systems may not be good for catalysis. For instance, resistance brought on by restricted development could prevent biofilms from producing molecules connected to growth. Additionally, restricted efflux or degradation of hazardous substrates would have a detrimental impact on production.

The Function of Gene Transfer in Biofilms in Bioremediation

In both natural and artificial contexts, many bacterial biofilms efficiently and often transmit genes. A population's capacity to adapt to and take advantage of changing environmental circumstances is impacted by gene transfer. In biofilms, gene transfer happens via two different processes: conjugation and trans-formation. Additionally, the transfer of catabolic genes in both natural and in vitro biofilms has shown significant promise for bioremediation procedures. In biofilms, many gene carriers known as mobile genetic elements are employed for gene transfer. The development of new catabolic pathways and bioremediation techniques may be aided by the horizontal exchange of catabolic genes across bacteria in their metabolic pathways. In situations where degradation is constrained by low biomass and a dearth of degradative genes, the transfer of catabolic genes may be employed to maximise bioreactor performance[9].

MFC Function and External Electron Transfer in Biofilms

Electro-chemically active biofilms are a tiny subset of biofilms that may directly exchange electrons with electrodes and are mostly generated by dissimilatory metal-reducing bacteria. EABs transmit electrons extracellularly through membrane proteins, conductive nanowires, or redox mediators made by microbes. Redox mediators produced by microorganisms are capable of repeated oxidation and reduction, which helps in the transfer of electrons between electrode and biofilm. Phenazines, compounds associated with quinones, and flavins are a few examples of redox mediators generated by microbes. At the surface of a conductive electrode, EAB-forming bacteria with direct extracellular electron transfer ability may change their metabolism from a soluble electron source or acceptor to a solid electron donor or

acceptor. The significance of bacterial cell surface appendages in the transport of electrons between biofilms and electrodes has recently been documented.

Microorganisms are a potent substitute for manmade chemicals in the biocontrol of plant diseases. According to recent research, bacteria must be in the biofilm phase in order to function as a biocontrol agent. According to this process, a specific strain's biofilm on plant surfaces may inhibit a disease without having a negative long-term impact on the rest of the local microbial community. Few biocontrol agents are now commercially accessible; the lack of understanding of the biological control system and the challenge of coming up with a suitable formulation are the main causes of the technology's delayed development. The majority of biocontrol agents have shown promise in laboratory tests, but scaling up production, ensuring formulation efficacy, and stability remain challenging tasks. For instance, field application of viable formulations of fluorescent Pseudomonads encounters a significant challenge. Sporulating Gram-positive bacteria like Streptomyces and Bacillus may be able to aid with the issue of product formulation. These bacteria produce spores that are heat- and desiccation-resistant and are easily transformed into stable goods like dry powder[10].

Bacterial Biofilms with Gramme Positives as Biocontrol Agents

In the plant rhizosphere, or the biologically and chemically active region of soil around the plant root, Bacillus subtilis and Bacillus cereus are often detected. Both B. subtilis and B. cereus operate as biocontrol agents and encourage plant development in the rhizosphere. While B. cereus has been used commercially for reliable biocontrol of various phytopathogens, especially oomycete pathogenic fungi, B. subtilis has proven highly effective against a variety of pathogenic bacteria including Erwina, Pseudomonas, and Xanthomonas strains.

After colonising the roots, B. subtilis and B. cereus produce large and durable biofilms. Surfactin, a powerful biosurfactant that is essential for preserving the aerial structure of biofilms, is one of the several antibacterial compounds produced by B. subtilis biofilm. Zwittermicin A and kanosamine, two antibiotics produced by B. cereus biofilm, aid in the biocontrol of lucerne damping. The simplicity of product formulation and storage has contributed to the commercial success of these organisms as biocontrol agents.

CONCLUSION

In conclusion, molecular community analysis methods have revolutionized the study of microbial communities by enabling researchers to identify and characterize microorganisms without the need for cultivation. These methods have facilitated the study of microbial community structure, diversity, and function, and have provided insights into the roles of microorganisms in biogeochemical cycles, bioremediation, and human health.

Molecular community analysis methods have their advantages and limitations. They can provide a more comprehensive view of microbial communities than traditional culture-based methods, but they may not accurately reflect the true diversity of microorganisms present in a sample. Furthermore, the interpretation of molecular community analysis data can be complex, and bioinformatics expertise is required for data analysis.

Continued advancements in molecular community analysis methods, such as the development of new sequencing technologies and analytical tools, will lead to further insights into microbial community structure and function. Additionally, interdisciplinary collaborations between microbiologists, ecologists, and bioinformaticians are necessary to fully realize the

potential of these methods for understanding the role of microorganisms in the environment and for developing biotechnological applications. Overall, molecular community analysis methods have transformed the field of microbial ecology and will continue to do so in the future.

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CHAPTER 7

ROLE OF CORROSION IN MICROBIAL TECHNOLOGY

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ABSTRACT:

The prevention of corrosion by biotic or abiotic inhibitors is known as corrosion inhibition. Corrosion inhibition has an electrochemical character, which is the same basic process as corrosion. The creation of a straightforward barrier on the metal is seldom all that corrosion prevention mechanisms entail. Zuo has examined current developments in the control of corrosion by helpful bacterial biofilms and proposed four alternative ways. Below, they are covered in further depth. Biofilm Corrosion Inhibition via Oxygen Removal.The degree of corrosion inhibition caused by bacteria that form biofilms is a common phenomena and depends on the kind of biofilms formed. Additionally, it is recognised that a deeper biofilm layer results in a higher decline in corrosion. Additionally, bacterial metabolic processes help biofilms remove oxygen from the air.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Corrosion Inhibition by Antimicrobial-Secreting Biofilms Corrosion control methods using regenerating biofilms are sometimes referred to as corrosion control employing biofilms that secrete antimicrobials. By using this method, the biofilm may produce antibacterial substances. Additionally, by preventing them from migrating into bulk fluids, EPS aid in maintaining comparatively greater local antibacterial concentrations. Genetically engineering B. subtilis and B. brevis to form biofilms that secrete antimicrobials, Jayaraman et al. discovered that these biofilms significantly decreased corrosion rates and hindered the development of the sulfate-reducing bacteria that cause corrosion. According to a field trial done by Zuo et al., B. brevis biofilm protection against a variety of corrosive bacteria reduced the rate of mild steel corrosion by a factor of two to ten[1], [2].

Corrosion Prevention via Secreting Biofilms Anti-corrosion Agents

Since corrosion inhibitors are produced inside and close to biofilms, they are the perfect vehicle for delivering them to metal surfaces. The effectiveness of polyaspartate or -polyglutamate as corrosion inhibitors was studied by Mansfeld et al. By forming an aluminum/anionic peptide combination, polyaspartate and polyglutamate both chelate metals, preventing the metal surface from uniform corrosion.

Prevention of Corrosion by Use of Protective Layers

A passive oxide product created during corrosion that is confined in the biofilm matrix might serve as protective layers. By forming an iron oxide/iron phosphate layer inside the biofilm matrix, aerobic Pseudomonas cichorii was able to prevent mild steel from corroding, according to Chongdar et al. Bacillus mycoides, a Gram-positive, nonmotile soil bacterium,

was identified by Juzeliunas et al. It enhances the aluminum's charge transfer resistance and prevents corrosion. Technologies Based on Biofilm Reactors for Biofilm[3]. Biofilms can be used in a variety of reactor types, including membrane-aerated biofilm reactors, upflow anaerobic sludge blanket reactors, packed bed reactors, trickling bed reactors, airlift reactors, and continuous stirred tank reactors. The fundamental benefit of biofilm reactors over traditional suspended biomass reactors is their high response rates.

A CSTR is a vertically baffled tank with a mechanical stirrer attached to it. It receives influent constantly, and treated effluent is drawn out of the reactor at the same pace. For the formation of biofilms, the PBR employs a range of packings, including polymers, ceramic, glass, and natural materials. The TBR uses counterflow of gas and liquid via packing material or beds, in contrast to the PBR. TBRs are used for water filtration, ore leachate treatment, and large-scale vinegar manufacturing. They are also used as biofilters for gas odour removal and cleaning. Microbial cells develop into active biofilms surrounding adsorbent ppapers in FBRs. FBRs are capable of sustaining large biomass volumetric concentrations and high substrate conversion rates. In FBRs, liquid is forced through ppapers beyond the "minimum fluidization velocity," which causes ppapers to be lifted off their fixed bed. Compared to PBRs or CSTRs, FBR may be used for significantly longer periods of time without suffering obstruction.

Wastewater and industrial effluents are treated anaerobically in UASB reactors, which are based on the usage of granular biofilm ppapers. Provisions are built at the top of the reactor for gases to escape and sludge ppapers to collect at the bottom of the reactor. It is necessary to have an acceptable influent distribution in order to increase the process efficiency of UASB reactors. EGSB reactors are a consequence of the utilisation of effluent recirculation in conjunction with a taller reactor. EGSB reactors are also utilised to treat wastewater industrial effluents anaerobically. Circular mixing is performed via gas injection in ALRs. As a consequence, at the same rate of airflow, there is less shear and more active recirculation.

A riser and a downcomer, two concentric tubes, are present in ALRs. At the bottom of an IC reactor, influent is pumped and mixed with granular anaerobic biomass. The majority of the organic components are transformed into meth- ane and carbon dioxide in the bottom reactor compartment. The self-regulating IC reactor provides significant system operating advantages, resulting in lower operational costs and higher productivity and dependability. The MABR employs membranes that are supplied with oxygen and immobilised microbial biomass. MABRs reduce volatile organic compound losses and make it simple to manage how much oxygen gets through the membranes and into the biofilms. Aerobic microbial strains thrive in the gradient of dissolved oxygen between the membrane and the biofilm, which also reduces foaming in the reactor from surfactant production. Additionally, MABRs are a desirable choice for pollutant bio-degradation in high-strength wastewaters due to improved oxygen penetration.

Wastewater and Waste Gas Treatment using Biofilm Reactors

There has been a thorough evaluation of the use of several kinds of biofilm reactors in wastewater treatment elsewhere. An appealing and eco-friendly alternative to physicochemical approaches for waste gas treatment is biological treatment. The three main bioreactor designs typically used for waste gas treatment are the biofilter, trickling biofilter, and bioscrubber. A biofilter is made consisting of a filter bed that serves as both a source of nutrients and support for the active microorganisms. The biofilms that surround the individual pieces of biofilter media are exposed to waste air. In contrast, waste gas is continually supplied with a liquid medium to the biofilm that has developed on the packing

material in trickling biofilters. The bioscrubber is mostly used to remediate waste gases that include high levels of water-soluble contaminants[4].

DISCUSSION

Reactors for Biofilm in the Bioremediation Process

Microorganisms are used in bioremediation, a new in situ approach that is effective and affordable for cleaning up environmental toxins. Because of their high microbial biomass and capacity to immobilise substances via biosorption, bioaccumulation, and biomineralization, biofilms are ideally suited for the treatment of resistant or slowly degrading substances. Biosurfactant synthesis and chemotaxis, two physiological traits of microbes, promote the bioavailability and breakdown of hydrophobic substances.

Hydrocarbon Bioremediation

Numerous chlorinated aromatic compounds are carcinogenic even at very low concentrations, making them common pollutants of soil and groundwater. The various bioreactor types employed in the bioremediation of hydrocarbons. Using a rotating perforated tube biofilm reactor with a mixed microbial bio- mass enriched with DCP-degrading Pseudomonas putida, Kargi and Eker demonstrated 100% degradation of 2,4-dichlorophenol. According to Johnsen et al., microbial exopolymers serve as a catalyst for the accumulation of polycyclic aromatic hydrocarbons in bacterial biofilms. After that, the biofilm community breaks down the accumulated PAHs when it is starved. Atrazine-degrading genes were inserted into the biofilms of Acinetobacter sp. BD3 by Perumbakkam et al., creating a biofilm-mediated method of atrazine degradation. Therefore, a combination of genetic engineering of microorganisms and optimisation of physi- cochemical parameters and substrate concentrations in bioreactors is of utmost importance for developing bioremediation strategies. Improving strains by engineering metabolic pathways and enzymes involved in degradation or by increasing the number of copies of degrada- tive genes could further enhance biofilm-mediated bioremediation.

Immobilisation, biosorption, and concentration of heavy metals by bioremediation reduce potential risks. Many findings point to the involvement of biofilm EPS in metal precipitation, such as Citrobacter sp. N's accumulation of polycrystalline NaUO2PO4 and Desulfobacteraceae members' production of sphalerite in a natural biofilm. Metal precipitation procedures may be made better by creating superior strains with particular metal-binding capabilities by production of metal-chelating proteins and peptides.

Reactors for Biofilm in Productive Biocatalysis

An efficient and green method for producing chemicals for commercial use is biocatalysis. Under typical circumstances, biocatalysts such as bacteria, fungus, and their enzymes may be created from renewable resources, are biodegradable, and catalyse processes with great selectivity. The key obstacles to effective and efficient biocatalysis are cell retention during continuous bioreactor operation and long-term cell survival, and microbial biofilms provide answers to both issues. Biofilms naturally adapt to the immobilisation matrix they make for themselves and may survive there. This provides the opportunity for continuous reactor usage and a considerable cost savings over traditional systems. Numerous biofilm techniques have already been successfully used commercially over the last several decades because to their reliability and simplicity of use. One instance is the use of acetic acid bacteria in the commercial production of vinegar. The viability of using biofilm reactors to turn agricultural products like starch, sugars, and glycerol into alcohols or organic acids was examined by

Qureshi et al. Wang and Chen have analysed the various options for producing biofilm-based biofuel. The potential of biofilms to produce other valuable compounds has been the subject of more field investigation[5].

The subject of contemporary research has been biofilm processes involving hazardous substrates and harmful byproducts. According to Li et al., Zymomonas mobilis biofilms were able to manufacture benzyl alcohol in a continuous biofilm reactor and were more resistant to the hazardous substratum benzaldehyde than free-floating cells of the same strain. Styrene is a physiologically problematic chemical since it is volatile, weakly water-soluble, and poisonous to cells. Gross et al. developed a tubular biofilm reactor for bioconversion of styrene to -styrene oxide. The promise of biofilm applications for industrial catalysis has been shown in a number of different studies that have successfully used biofilms in productive catalytic processes without biocatalyst degeneration or contamination over many months.

Microbial catalysts are used in microbial fuel cells (MFCs), which turn chemical energy stored in reduced carbon molecules directly into electrical energy. A standard MFC is made up of two chambers, an anode and a cathode, separated by a cation exchange membrane, however the design of an MFC primarily depends on the application. At the anode, electroactive biofilms oxidise the substrate and release protons and electrons. While the protons are carried across the cationic membrane, the electrons are delivered to the cathode through an external circuit. A terminal electron acceptor is decreased at the cathode[6].

Environmental factors like the presence of oxygen and the availability of a readily degradable carbon source have an impact on an MFC's power output. Various substrates, such as glucose, acetate, sucrose, ethanol, butyrate, glutamate, and wastewater, are used to power MFCs. The designs of MFCs are evolving quickly; for instance, the cationic membrane has been eliminated to minimise costs, and both anodic and cathodic material are being researched to boost the electroactivity of Marine MFCs.

As illustrated in Fig. 3.2b, marine MFCs generally consist of a graphite anode entrenched in anaerobic marine sediments that is electrically coupled to a cathode established in the waters below that is aerobic. The continual regeneration of microbial electrode catalysts occurs in marine MFCs as a consequence of environmental processes such as the settling of dead phytoplankton and/or vegetative debris. MFCs used in marine sediments exhibit long-term sustained current output. For the first time, Tender et al. showed how marine MFC may be used as a workable substitute for batteries in low-power applications like buoys and maritime sensors.

Drainage MFCs

MFCs may be effectively used with a wide range of substrates, including those that are more challenging to process, such household wastewater, swine manure slurry, landfill leachate, and meat-packing wastewater. Researchers have obtained 0.7 V individual cell voltages with power densities ranging from 2.0 mW/m2 of anode surface area to 2,0 mW/m2 of anode surface area, depending on the MFC architecture, sub- strate, anodophilic bacteria, and operation parameters utilised. The integration of MFCs will significantly reduce the cost of treating wastewater.

Agricultural MFCs

The use of MFCs in agricultural fields to produce electricity from cultivated plants is an intriguing additional use. Through the in-situ oxidation of rice plant rhizodeposits, electrical current was produced. In the presence of actively growing plants, a sediment MFC's electrical

power production was shown to be seven times greater. This procedure provides the possibility of non-destructive light-driven power production from live plants. The oxidation of chemicals generated from plants may be responsible for the sustainable power generation up to 0 W/h. Small quantities of electricity may be produced with this technique in isolated, off-grid settings[7].

MFCs that are Photonic

Microalgae and EABs have been combined in this relatively new technique to produce 0 mW/m2. Due to their low nutrient and organic carbon requirements, microalgae are especially intriguing for energy conversion processes. Rosenbaum et al. have recently examined the five methods that combine photosynthesis with MFCs.

Use Cases for MFCs

The use of MFCs in a variety of sectors, such as wastewater treatment, bioremediation, metal oxidation, and the manufacture of biosensors and biohydrogen, is attracting growing attention from researchers. MFCs are used to remove oxidizable material from home and industrial wastewaters. The studies that have been published show how effective MFCs are at treating wastewater.

It is realistic to predict that MFCs will eventually replace complicated and energetically unsustainable methods now in use for wastewater treatment, even if scaling up and long-term viability of MFCs remain a challenging problem. According to recent reports, the biocathode in MFCs may extract significant energy from wastewater contaminants. In wastewater-fed MFCs, biocathodes may function as effective catalysts for oxygen reduction and a workable replacement for abiotic systems. Holmes et al. discovered that biological processes like ammonia oxidation and denitrification included a biocathode system of benthic MFCs. In separate anodic and cathodic chambers, Virdis et al. reported an MFC design for simultaneous nitrification and denitrification. For nitrification to take place, the anode effluent was aerated externally, and the nitrified effluents were then sent to the cathode for denitrification.

Leptothrix discophora, a manganese-oxidizing bacterium, was used by Rhoads et al. to convert Mn2 into MnO2 by releasing two electrons to oxygen. Li et al. reported effective chromium removal with a maximum power density of 1,0 W m at a Columbic efficiency of% utilising graphite paper as a cathode. The system's ability to lower energy costs for hydrogen production was made possible by the incorporation of MFC in the water electrolysis process. Microbial electrolysis cells are the name given to this technique. In a conventional MEC, the current generation is pushed and a modest voltage is supplied between the anode and the cathode, leading to hydrogen creation at the cathodic side owing to proton reduction. Many organic molecules cannot be used as a substrate for fermentative hydrogen generation because of a thermodynamic barrier. They are nonetheless applicable to MECs since in these systems, a negligible external voltage is utilised to break through the thermodynamic barrier.

Since ancient times, bacteria have been employed to ferment foods and alcoholic beverages. In recent years, however, microbes have come under close scientific investigation due to their potential for curative and preventative benefits in humans. This research resulted in the coining of the word "probiotics." The typical microflora of most animals' intestines includes lactic acid bacteria. They function as an immunomodulator in both humans and other animals and have a significant role in both. LAB are beneficial for better digestion and nutrient absorption, as well as for the treatment and prevention of illness. LAB, or Lactobacillus acidophilus, L. bulgaricus, L. casei, L. plantarum, L. rhamnosus, etc., are examples of

probiotic bacteria. It is a recent discovery in vaccine formulation to use these live bacteria to stimulate an immune response or to deliver a vaccine component. Live bacterial vaccines have the benefits of mimicking the natural infection, having inherent adjuvant qualities, and being administered orally. Currently, potential oral vaccination candidates include both pathogenic and nonpathogenic food-related microorganisms.

Since the inception of vaccination, live vaccines have been crucial. Due to our improved knowledge of immunology and the availability of numerous procedures that make the development of safer live vaccines, the idea of live vaccinations has significantly garnered interest over the past 20 years.

Lilley and Stillwell used the word "probiotics" to describe a chemical that encourages the development of other bacteria. Recent changes to the definition of this word have limited its meaning to "a viable microbial agent that, when used for other organisms like animals or people, benefits the host by improving the balance of the natural microflora." Metchnikoff published the first study on the probiotics' positive effects in the early 1900s, documenting the advantages of fermented milk in people. It has also been suggested that ingesting living bacteria from sour milk may assist to regulate the gut microbiota[8].

Probiotic bacteria need to be resilient to intestinal peristalsis, bile acid, pH, proteolytic enzymes, and antimicrobial peptides in order to live in the mouth cavity. Live vaccines made from pathogenic and nonpathogenic food-related microbes are now being tested.Lindberg's analysis of the background of live bacterial vaccines is outstanding. The first live bacterial vaccination was administered subcutaneously in Spain in and included attenuated Vibrio cholerae. Field tests with a more potent parenteral V. cholerae vaccine were conducted in India after that research.

CONCLUSION

In conclusion, the role of microorganisms in corrosion is significant, and their impact on material degradation cannot be overlooked. Microorganisms can accelerate corrosion through a variety of mechanisms, including biofilm formation, production of corrosive metabolites, and modification of the local environment. Microbial corrosion is a complex phenomenon that is influenced by a variety of factors, including microbial community composition, environmental conditions, and material properties. As such, effective management and prevention of microbial corrosion requires a multi-faceted approach that involves understanding the underlying microbial mechanisms, optimizing material selection and design, and implementing effective control strategies.

Microbial technology has great potential to address the challenges associated with microbial corrosion. Biocides, enzymes, and biofilm inhibitors are among the various microbial-based technologies that have been developed to mitigate microbial corrosion. Furthermore, advances in microbial genomics and metagenomics are enabling the identification and characterization of microorganisms involved in corrosion processes, and providing insights into the mechanisms by which they contribute to material degradation.

The study of microbial corrosion has advanced our understanding of the complex interactions between microorganisms and materials, and has led to the development of innovative microbial-based technologies for managing and preventing corrosion. Continued research in this area, as well as interdisciplinary collaborations between microbiologists, material scientists, and engineers, will lead to further advancements in microbial technology for corrosion control and prevention.

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CHAPTER 8

IMPORTANCE OF PROBIOTIC BACTERIA

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ABSTRACT:

Probiotic bacteria are safe and have GRAS status in both human and animal systems. The ability of cells to produce secondary metabolites and enzymes, adhesion qualities that help them stick to the epithelium, factors that affect the strain's survival, interactions with the host body, particularly in terms of preventing pathogenic microbes, are all taken into account when evaluating a probiotic strain's safety. LAB, or Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Aerococcus, Bifidobacterium, and Weissela, are Gram-positive bacteria. The probiotic strain most often employed is Lactobacillus. It has been recommended to consume Lactobacillus as a probiotic because to its advantages for disease resistance, including the improvement of the immune system and resistance to malignant growth and other infectious disorders. Immune system dysfunction is widely known to occur with ageing, stress, viral illnesses, and malnutrition..

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Immunomodulation may treat the aforementioned inadequacies by using the appropriate natural and synthetic substances and/or products. However, the negative side effects of the immunostimulatory medicines now on the market are frequent. To improve the health of huge populations with compromised immune systems, it would be crucial to produce natural compounds with immunomodulatory capabilities that are free of adverse effects. Although vaccination is a reliable and affordable method of avoiding infectious illnesses, it is costly and risky to provide mass vaccinations since the vaccines that are now available must be injected. When compared to parenteral vaccinations, oral vaccines offer a number of advantages, but their application has been restricted to infections that are transmitted via the mucous membranes. It has not been acknowledged that they may potentially control non-mucosally transmitted illnesses[1].

A significant obstacle in the fight against infectious illnesses is the creation of oral live vaccinations against pathogens. In a big population with limited healthcare resources, the straightforward administration by mouth into the host is an alluring substitute for lengthy pharmacological treatments. An appealing vaccination technique involves using bacteria to trigger an immune response to either the bacteria or a component of the bacterium. Live vaccines have the advantages of mimicking the disease or antigen, having adjuvant qualities, and being able to be given orally. Oral vaccinations may also be derived from pathogenic and nonpathogenic food-related microorganisms. The idea of using live, attenuated microorganisms as an oral vaccination is not new. As a typhoid vaccine, previously attenuated Salmonella enterica serovar of Typhi given as an acid-resistant capsule has already been commercialised. The foundation of a commercial cholera vaccine is attenuated V.

cholerae. However, it is possible to transfer heterogonous antigens, or antigens against diseases other than themselves, utilising attenuated bacteria[2].

Salmonella typhi, Shigella flexneri, Listeria monocytogenes, V. cholerae, and Escherichia coli are only a few pathogenic and nonpathogenic bacteria that have derivatives that may be utilised as live vaccines. Other nonpathogenic bacteria include Lactobacillus and Bifidobacterium.

These probiotic bacteria specifically target areas of the human immune system that are thought to be inductive, such as mucosal surfaces and macrophage antigen-presenting cells. Less LAB is used to administer the vaccine than is used to transmit attenuated pathogens. These are secure, and the availability of genetic resources for recombinant gene expression in laboratory settings makes them enticing as potential vaccine candidates. The vaccine delivery to APC may be less successful than for invasive bacteria since LAB are not harmful. Nevertheless, with a number of LAB, particular immune responses have been attained. Probiotics are said to improve the maintenance of native microflora, prevent infectious diseases and allergies, lower serum cholesterol levels, have anticancer activity, stabilise the gut mucosal barrier, act as immune adjuvants, lessen symptoms of inflammatory bowel disease, and improve the digestion of lactose in lactose-intolerant hosts, among other health benefits.

GALT Immunity and Probiotics

A complex network of signals, various interactions between commensal and foreign antigens, and interactions with the host cell are necessary for the gut mucosal immune system to operate. In addition to mucous-producing cells like goblet cells and Paneth cells, which emit antimicrobial peptides and create cryptidins or defensins, these host cells also comprise epithelial cells, macrophages, dendritic cells, and other cells from the nonspecific barriers[3].

The epithelial cells and GALT must interact with nonpathogenic probiotic microorganisms. Because they release chemokines and cytokines that regulate immune cells in both specific and nonspecific immunological responses, mucosal epithelial cells play a crucial role in coordinating defence systems. The inflammatory reaction must be under control and cannot be brought on by naturally occurring commensal bacteria in the intestine. Luminal secretory IgA is produced by probiotic microorganisms. The most notable effect of probiotic bacteria and fermented milk yoghurt was an increase in the number of IgA-producing cells. IgA has an undisputed physiological significance in the mucosal surface. Some probiotic organisms may boost the mucosal and overall immune response.

Probiotic microorganisms have the ability to trigger the immune system's network of signals in the gut mucosa, which depends on bacterial interaction with the gut's immunological and epithelial cells. LAB correlate with the many routes that interact with antigens in the digestive system. These bacteria engage in interactions with the related immune cells, gut epithelial cells, and M cells in Peyer's patches. Cytokines that are released by cells may change how the immune system responds. Tumour necrosis factor alpha, gamma interferon, interleukin-, and other cytokines, as well as proinflammatory mediators like reactive nitric oxide, are produced as a result of bacterial vaccine vectors, enhancing early innate immunity and fostering an environment that is conducive to antigen presentation[4].

More than two thirds of the immune system's activity is attributed to mucosal epithelial cells, which provide an effective barrier to keep environmental pathogen antigens out of the host mucosal immunity. The quantity of immunoglobulins generated at these locations, the size of the mucosal tissues, and the number of immune-competent cells are all taken into account in

these conclusions. The presence of secretory immunoglobulins, or s, and specialised cellular flagel-lated bacteria, including commensals, separate mucosal immunity from systemic immunity. In order to connect the humoral and cell-mediated immune responses, these substances cause epithelial homeostatic chemokine responses that draw immune cells of the innate immune system to the gut epithelium and lamina propria of the intestinal mucosa.

TLR 2 and TLR 4 in particular may have their signals activated by LAB's recognition of TLR. These peptides serve as the primary receptor for the bacterial species' lipopolysaccharides. Lipoteichoic acid, peptidoglycan, and/or lipoproteins and/or lipopeptides are some of the additional bacterial components that TLR 2 mediates signals from. However, there is disagreement concerning where exactly these receptors are located in the intestinal epithelial cells. TLR signals are crucial for maintaining intestinal barrier functions as well as for responding to infections. The interaction between the probiotic bacteria and the intestinal mucosa must have a number of effects for it to be used as an immunity booster. Beneficial effects from probiotic bacteria may be transmitted via colonisation and/or the production of certain bioactive substances. These actions result in direct control of epithelial cell activities, including cytokine and chemokine release, as well as fortification of the intestinal barrier. In addition to inducing innate and adaptive immunity, this causes monocytes and macrophages to produce cytokines. The epithelial cells and other immune cells get more signals as a consequence, and native T cells are given microbial antigens in the Peyer's patches and mesenteric lymph nodes. This triggers an IgA antibody-mediated mucosal response to control the bacteria and stop it from growing too much and spreading outside of the MLN.

It has been shown that nonpathogenic bacteria or their pieces may be taken up by macrophages or dendritic cells in the lamina propria. This is made feasible by the direct sampling of luminal antigen for dendritic cells via TLRs and CD-6 mannose receptors. These bacteria have the ability to be eliminated or carried to the mesenteric lymphatic node, where they interact with T and B cells to either inhibit T cells or generate certain mucosal IgA. Since they alter the gut flora, probiotic microorganisms have long been thought to be beneficial. Probiotic Lactobacillus strain oral vaccines are effective against a wide range of bacterial, viral, and protozoal illnesses.

Malaria

Most Plasmodium species include the surface protein layer known as merozoite surface protein 1. MSP1 has being investigated as a key candidate for a malaria vaccine. Plasmodium's high-level expression of MSP1 during the asexual stage is crucial for the parasite's entrance into RBCs. Proteolytic cleavage of MSP1 may produce five fragments. It involves two steps. On the surface of mature merozoites, there is a carboxyl-terminal -ku segment.

DISCUSSION

By applying the proper natural and synthetic substances and/or products, immunomodulation may be able to address the aforementioned deficiencies. However, the currently available immunostimulatory medications often have unwanted side effects. It would be essential to develop naturally occurring substances with immunomodulatory properties that are devoid of side effects in order to enhance the health of large populations with weakened immune systems.

Although vaccination is a trustworthy and economical way to prevent infectious diseases, administering mass immunisations is expensive and dangerous since the vaccines that are now available must be administered. Oral vaccines provide a variety of benefits over

parenteral immunisations, but they have only recently been used to prevent illnesses that spread via the mucosal membranes. The possibility that they may manage non-mucosally transmitted diseases has not been recognized [5].

The development of oral live vaccines against pathogens is a crucial barrier in the battle against infectious diseases. The direct delivery by mouth into the host is an enticing replacement for protracted pharmacological therapies in a large population with little healthcare resources. Using bacteria to stimulate an immune response to either the bacteria or a component of the bacteria is an interesting immunisation method. The ability to imitate the illness or antigen, have adjuvant properties, and be administered orally are all benefits of live vaccines. Additionally, both pathogenic and nonpathogenic food-related microbes may be used to provide oral vaccines.

Oral immunisation with live, attenuated microbes is not a novel concept. Previously attenuated Salmonella enterica serovar of Typhi administered as an acid-resistant capsule has already been commercialised as a typhoid vaccine. Attenuated V. cholerae serves as the basis of a commercial cholera vaccine. However, using attenuated microorganisms, it is feasible to convey heterogonous antigens, or antigens against illnesses other than their own. Escherichia coli, Salmonella typhi, Shigella flexneri, Listeria monocytogenes, V. cholerae, and other pathogenic and nonpathogenic bacteria are only a few examples of those with derivatives that may be used as live vaccines. Bifidobacterium and Lactobacillus are two more nonpathogenic bacteria[6].

Targets of these probiotic bacteria include mucosal surfaces and macrophage antigen-presenting cells, which are regarded to be inductive components of the human immune system. The vaccine is delivered with a smaller amount of LAB than attenuated pathogens are. These are safe and appealing as prospective vaccination candidates because to the availability of genetic resources for recombinant gene expression in lab settings. Since LAB are not hazardous, the vaccine delivery to APC may be less effective than for invasive bacteria. However, specific immunological responses have been obtained with a variety of LAB. Probiotics have been linked to a number of health benefits, including improved native microflora maintenance, the prevention of allergies and infectious diseases, lowered serum cholesterol levels, anticancer activity, stabilisation of the gut mucosal barrier, immune adjuvant effects, a reduction in the symptoms of inflammatory bowel disease, and improved lactose digestion in lactose-intolerant hosts.

The gut mucosal immune system requires a complicated network of signals, many interactions between commensal and foreign antigens, and interactions with the host cell. These host cells include epithelial cells, macrophages, dendritic cells, and other cells from the nonspecific barriers in addition to mucous-producing cells such goblet cells and Paneth cells, which release antimicrobial peptides and produce cryptidins or defensins. The nonpathogenic probiotic microbes must interact with the epithelial cells and GALT. Mucosal epithelial cells play a critical part in the coordination of immune defence mechanisms because they secrete chemokines and cytokines that control immune cells in both targeted and nonspecific immunological responses. Natural commensal bacteria in the colon cannot trigger the inflammatory response, which must be under control. Probiotic microorganisms generate luminal secretory IgA.

The probiotic bacteria's and fermented milk yoghurt's most striking result was a rise in the number of IgA-producing cells. Unquestionably, IgA has a physiological role on the mucosal surface. Some probiotic organisms may strengthen the immune system's mucosal and general defences. Probiotic microorganisms have the power to activate the gut's mucosal network of

immune signals, which is dependent on bacterial contact with immune and epithelial cells. LAB are related to the many ways that the digestive system interacts with antigens. These bacteria interact with Peyer's patches' M cells, gut epithelial cells, and immune cells that are relevant to them. Cells may secrete cytokines that can alter how the immune system reacts. Bacterial vaccine vectors enhance early innate immunity and foster an environment that is conducive to antigen presentation by inducing the production of cytokines such as tumour necrosis factor alpha, gamma interferon, interleukin-, and other cytokines, as well as proinflammatory mediators like reactive nitric oxide.

Mucosal epithelial cells, which serve as an efficient barrier to keep environmental pathogen antigens out of the host mucosal immunity, are responsible for more than two thirds of the immune system's activity. These results take into consideration the amount of immunoglobulins produced at these sites, the size of the mucosal tissues, and the number of immune-competent cells. Mucosal immunity and systemic immunity are distinguished by the presence of secretory immunoglobulins, or s, and specialised cellular flagel-lated microorganisms, including commensals. These chemicals trigger epithelial homeostatic chemokine responses that attract immune cells of the innate immune system to the gut epithelium and lamina propria of the intestinal mucosa in order to link the humoral and cell-mediated immune responses.

The signal activation of TLR 2 and 4 in particular may be caused by LAB's identification of TLR. These peptides operate as the main receptors for the lipopolysaccharides of the bacterial species. Other bacterial components that TLR 2 mediates signals from include lipoteichoic acid, peptidoglycan, and/or lipoproteins and/or lipopeptides. Regarding the precise location of these receptors in the intestinal epithelial cells, there is dispute. TLR signals are essential for preserving the integrity of the intestinal barrier and recognising and reacting to infections. To be effective as an immune booster, the probiotic bacteria's contact with the intestinal mucosa must have a variety of outcomes. Probiotic bacteria may spread their beneficial effects via colonisation and/or the synthesis of certain bioactive compounds. Through these processes, the intestinal barrier is strengthened and epithelial cell activities, such as the release of cytokines and chemokines, are directly controlled. This triggers the production of cytokines by monocytes and macrophages in addition to innate and adaptive immunity. As a result, the epithelial cells and other immune cells get more signals, and the Peyer's patches and mesenteric lymph nodes provide native T cells with microbial antigens. In order to regulate the bacterium and prevent it from being out of control and spreading outside of the MLN, this causes an IgA antibody-mediated mucosal response[7].

It has been shown that dendritic cells or macrophages in the lamina propria may take up nonpathogenic bacteria or their fragments. This is made possible by TLRs and CD-6 mannose receptors directly sampling luminal antigen for dendritic cells. The mesenteric lymphatic node is where these bacteria may be removed or transported, where they interact with T and B cells to either suppress T cells or produce specific mucosal IgA. Probiotic bacteria have long been regarded to be advantageous because they change the gut flora. Oral vaccinations using the probiotic Lactobacillus strain are beneficial against a variety of bacterial, viral, and protozoal diseases[8].

Malaria

The surface protein layer known as merozoite surface protein 1 is present on the majority of Plasmodium species. MSP1 is being researched as a potential malaria vaccine candidate. High-level MSP1 expression by Plasmodium during the asexual stage is essential for the parasite's invasion of RBCs. Five fragments of MSP1 might be produced via proteolytic

cleavage. There are two stages to it. Mature merozoites have a carboxyl-terminal -ku segment on their surface.

Scalability of the thin solid substrate fermentation technology, management of biomass, and large-scale extracellular enzyme secretion. Although biological methods are thought to be secure, affordable, sustainable, and environmentally friendly, they still have some drawbacks related to the cultivation of microbes, which takes a lot of time and is challenging in terms of providing the best control over the size distribution, shape, and crystallinity of nanoppapers. However, effective implementation of biological and biomimetic approaches for large-scale nanoppaper production for commercial applications can be achieved with careful strain selection and optimisation of conditions such as pH, incubation temperature and time, concentration of metal ions, and amount of biological material[9].

CONCLUSION

In conclusion, probiotic bacteria have shown great potential in improving human health by promoting a healthy gut microbiome and modulating the immune system. Probiotics have been found to alleviate various digestive disorders, such as irritable bowel syndrome and inflammatory bowel disease, and also have potential therapeutic applications for conditions such as allergies and respiratory infections. However, the efficacy of probiotics is highly strain-specific, and the mechanisms by which they confer their health benefits are still not fully understood. Additionally, the regulatory framework for probiotics varies between countries, and there is a need for standardized guidelines for their production and use.

Advancements in microbiome research and molecular biology techniques have enabled a better understanding of the mechanisms underlying probiotic activity, and have led to the development of more effective probiotic strains and formulations. Furthermore, there is growing interest in the potential of postbiotics, which are the metabolic products of probiotic bacteria, as a novel approach for improving human health.

In summary, the study of probiotic bacteria has led to promising developments in the field of microbial technology and human health. Further research is needed to fully understand the mechanisms by which probiotics confer their health benefits, and to optimize their use for specific health conditions. Standardized guidelines for production and use of probiotics will also be important for ensuring their safety and efficacy.

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CHAPTER 9

NANOPPAPER SYNTHESIS BY BACTERIA

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ABSTRACT:

One of the most often used natural resources for the manufacture of metallic nanoppapers is bacteria. The relative simplicity of their manipulation is the main factor in bacteria's propensity for nanoppaper formation. The creation of magnetite ppapers in siliceous materials by diatoms, gypsum and calcium layers by S-layer bacteria, and magnetotactic bacteria has been seen while investigating the mysteries of microbial synthesis of nanoppapers. Bioremediation, biomineralization, bioleaching, and biocorrosion are just a few biological applications that have made use of the interactions between metals and bacteria. Nanobiotechnology and the microbial creation of nanoppapers are two recent study areas that are rapidly expanding. Below we present the current state of study on the biosynthesis of certain frequently researched and used metal nanoppapers by various bacteria, actinomycetes, and cyanobacteria.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

For the production of silver nanoppapers, a wide range of techniques have been used, including photocatalytic reduction, chemical reduction, radiation-chemical reduction, metallic wire explosion, sonochemistry, polyol process, photoreduction, reverse micelle-based methods, matrix chemistry, and biological synthesis. It has also been reported that the silver-resistant bacterium Pseudomonas stutzeri AG9 isolated from silver mines can produce silver crystals. It has been discovered that this bacterium produces pyramidal and hexagonal silver nanoppapers up to 0 nm in size that are incorporated in the bacterial cell's organic matrix. In the same way, Morganella sp. When exposed to silver nitrate, RP-, an insect midgut isolate, formed extracellular crystalline nanoppapers that were around 5 nm in size. In Morganella species that are resistant to silver, three gene homologues have been found. The Morganella species' silE homologue exhibited nucleotide sequence resemblance to the previously known gene silE, which codes for a protein that binds silver in the periplasm. Additionally, Corynebacterium species cells[1].

Silver nanoppapers in the range of - nm may be produced by SH on the cell wall at °C within h using diamine silver complex +. The amino acid moieties that the proteins that bind silver supply act as nucleation sites for the development of silver nanoppapers. Additionally, face-centered cubic shaped silver crystals with the capacity to precipitate silver from an aqueous solution of silver ions have been discovered. Normal circumstances include tiny periplasmic silver-binding proteins binding silver at the cell membrane and launching incoming metals via efflux pumps to shield the cytoplasm from toxicity. a Bacillus sp. in the air. collected

metallic silver nanoppapers of 5-nm size in the periplasmic region of the cell and converted Ag+ ions to Ag0. Dried Aeromonas sp. cells have also been shown to create silver nanoppapers with a diameter of 6.4 nm. SH, which after 4 hours turned + into Ag0. These ppapers were stable for more than 6 months without aggregating or precipitating and were monodispersed, homogeneous in size. In addition, Enterobacteriaceae quickly produce silver nanoppapers in the range of.2 to 2 nm, with an average size of.5 nm, in their culture supernatants by converting Ag+ to Ag0. Piperitone partly reduced the reduction of silver ions when added, which indicated that nitroreductase enzymes were involved in the reduction process. The nonpathogenic bacteria B's culture supernatant is comparable. The extracellular synthesis of silver nanoppapers with a size of nm has been done using licheniformis. Barud et al. showed how Acetobacter xylinum culture membranes that had been hydrated and immersed in triethanolamine solution with silver ions could generate homogenous membranes that included bacteria and silver.

Recent research by Musarrat et al. describes the production of silver nanoppapers in the 5-nm size range generated by the industrially significant fungal strain KSU-, isolated from the roots of the date palm. Mycelia-free water extracts from mycelia suspended in water for hours have been shown to help produce stable, primarily mono-dispersed, and spherical nanoppapers when 1 mM silver nitrate is added, as shown by UV-visible spectroscopy, XRD, AFM, and TEM. The infrared spectrum showed that the medium included fungal proteins, which are probably to blame for the stability of the nanoppapers. As a result, after 2 hours, 3 hours, 4 hours, and 6 hours of incubation, bacteria from the panel display a time-dependent rise in the usual SPR bands in the UV-Visible absorption spectra of extracellularly synthesised silver nanoppapers at 0 nm. The inset illustrates how changes in SPR over time could be used as a natural bioresource for the quick, safe, and effective synthesis of AgNPs for the creation of next-generation nano-antimicrobials with a wide range of applications against multidrug-resistant microorganisms. This topic is covered in a different chapter part [2].

Nanoppapers of Gold

Gold nanoppapers have been widely synthesised using bacteria. Extremophilic Thermomonospora sp. was used by Ahmad et al. to show bacterial production of monodispersed gold nanoppapers. Enzymatic mechanisms are used to reduce AuCl4 ions and produce biomass. According to Konishi et al., mesophilic bacteria Shewanella was used in the manufacture of gold nanoppapers with H2 serving as an electron donor. Shiying et al. demonstrated that incubating bacterial biomass with an aqueous chlorauric acid solution within the pH ranges of 4.0 and 7.0 resulted in the production of spherical gold nanoppapers in the range of - nm by the bacterium Rhodopseudomonas capsulata. The pH of the solution has a key role in regulating the morphology of biogenic gold ppapers and the site of gold deposition in cells. Alkalotolerant Rhodococcus sp. created more intracellular monodispersed gold nanoppapers on the cytoplasmic membrane than on the cell wall as a result of enzymes present in the cytosol but not in the cell wall that reduced the metal ions.

Pseudomonas aeruginosa bacterial cell supernatants have been employed for the extracellular manufacture of gold nanoppapers as well as the reduction of gold ions. The precise process by which metal ions are reduced in organisms is yet unknown. However, gel electrophoresis measurements showed the existence of four distinct proteins with molecular weights ranging from to KDa, which may be in charge of the capping of the gold nanoppapers and reduction of the chloroaurate ions. According to reports, the bacteria Bacillus subtilis 8 may convert water-soluble Au3+ ions to Au0 and produce 5-nm octahedral nanoppapers inside the cell walls. Gold -thiosulfate complex Au2 has been destabilised to elemental gold of nm size in the bacterial envelope, releasing H2S as an end product of metabolism, using heterotrophic

sulfate-reducing bacterial enrichment from a gold mine. E. Chloroauric acid was bioreduced to Au0 by the coli DH5 enzyme, which caused the formation of predominantly spherical, but also some triangle- and quasi-hexagon-shaped nanoppapers on the cell surface. These nanoppapers that are attached to cells show promise[3].

applications for haemoglobin and other proteins in electrochemistry. Rhodobacter capsulatus, a photosynthetic bacterium with a greater biosorption capacity of HAuCl4 per gramme dry weight in the logarithmic phase of development, has also been shown to bioreduce trivalent aurum. It has been discovered that the biosorption and bioreduction of Au3+ to Au0 on the plasma membrane as well as outside the cell are carried out by carotenoid and NADPH-dependent enzymes that are incorporated in the plasma membrane and/or released extracellularly.

Nanoscale Magnets

It has been extensively shown that magnetotactic bacteria, which are Gram-negative bacteria with a variety of morphologies and are often found in both marine and freshwater sediments, are capable of producing magnetic nanoppapers. Greigite, pyrrhotite, and intracellular, membrane-bound magnetite are known to be produced by them. According to a study by Mann et al., the microaerophilic bacterium Aquaspirillum magnetotacticum creates organised, single-domain magnetite crystals with an octahedral prism shape and faces that are truncated by faces. In order to produce iron-rich magnetosomes, the marine magnetotactic bacteria MV-1 anaerobically bioreduced nitrous oxide and ferric quinate in sediments of an estuary salt marsh[4].

Each magnetite ppaper has a single magnetic domain and is a parallelepiped with nm-scale dimensions. Similarly, greigite, a single crystal of ferromagnetic iron sulphide that is allegedly coupled with nonmagnetic iron pyrite and aligned in chains, was intracellularly formed by magnetotactic bacteria isolated from brackish and marine sulfide-rich water and sediments. Approximately 10 nm-sized nanoppapers make up each chain. Most of the ppapers are asymmetrical, although others show considerable diffraction contrast and octahedral and cubo-octahedral symmetry. Magnetospirillum magneticum is a kind of bacteria that has shown the capacity to synthesise small, membrane-bound ferromagnetic ppapers made of magnetite or greigite. An internal phospholipid membrane surrounds these ppapers, generating magnetosome-like structures.

Each bacterial cell contains polydisperse nanoppapers ranging in size from 0 to. Crystallite and noncrystallite magnetic crystals both make up magnetosomes. In M. Magnetite nanoppapers have been discovered attached within the cells of the bacterium magnetotacticum, allowing the bacteria to passively orient themselves along the geomagnetic field. The cubo-octahedral crystal structure of each nanoppaper formed in the bacterial phospholipid membrane has a diameter of nm and a magnetic moment of 6 A m2. The magnetosomes of M also showed accumulation of magnetic iron mineral crystals into highly organised chain-like structures. The gryphiswaldense. Watson et al. showed how sulfate-reducing bacteria may produce magnetic iron sulphide nanoppapers as small as 2 nm. Furthermore, Actinobacter, a nonmagnetotactic bacteria, was used by Bharde et al. to produce magnetite nanoppapers. Microelectromagnets have been used to regulate the construction of magnetic nanoppapers inside of magnetotactic bacteria in fluid, as shown by Lee et al. Additionally, it has been revealed that the biomineralized magnetic nanocrystals in the multicellular magnetotactic bacteria Candidatus Magnetoglobus multicellularis allow it to interact with the geomagnetic field. When bacteria like A. neoform, they produce magnetite nanoppapers. Sulfate-reducing bacteria, the magnetotactic bacterium MV-1,

magnetotacticum, and M. M. magnetotacticum, M. Gryphiswaldense mostly displayed parallelepipeds, cubo-octahedra, hexagonal prisms, and octahedral prism morphologies in the size range of 2-0 nm [5], [6].

DISCUSSION

Nuclear Uranium Ppapers

Alteromonas putrefa- ciens cultivated in the presence of hydrogen as an electron donor and uranium as an electron acceptor converted uranium to uranium, according to reports on cell-free extracts of Micrococcus lactilyticus. Lovley et al.'s proof that G. When cultivated anaerobically with acetate and U acting as electron donors and acceptors, respectively, metallireducens GS- reduced soluble U to insoluble U and oxidised acetate to CO2. Desulfosporosinus sp., a Gram-positive sulfate-reducing bacterium isolated from sediments, has been discovered to reduce uranium to uranium, which precipitates to produce crystals of uraninite ranging in size from 1.5 to 2.5 nm and is coated on the cell surface. C-type cytochrome was discovered by Marshall et al. on the outer membrane of the dissimilatory metal-reducing bacteria S. In both cell suspension and periplasm, oneidensis MR-1 is engaged in the reduction of U, mostly with extracellular polymeric material as UO2-EPS[7].

Nanoppapers of Cadmium

Cadmium is largely utilised in the production of quantum dots, which are semiconductor metalloid-crystal structures with an average size of 2-0 nm and an atom count of 0-,0. QDs are very tiny, and as a result, they possess special optical and electrical characteristics that give the nanoppapers a brilliant, extremely steady, and "size-tunable" fluorescence. Due to their tiny size and vast surface area, QDs are extremely simple to functionalize with ligands for site-directed action. QDs might therefore be used for targeted drug administration, radio- and chemosensitization, cancer detection, and biological imaging at the cellular level. With CdSe and CdTe, which are most often utilised for biological applications, the active centre of the QD displayed as the core is composed of atoms from groups II to VI. The size-tunable fluorescence of QDs is a noteworthy property.

They are far more stable and much brighter than organic fluorophores. Since the fluorescence depends on ppaper size, a single light source may be used for both excitation and emission. This light source can be controlled to emit light at different wavelengths in the UV, visible, near-infrared, and mid-infrared spectrums. Because QDs are bigger than organic fluorophores, it is simple to add targeting groups to the surface of the nanopar- ticle. The optical, bioanalytical, and bioimaging applications of CdSe and CdTe are significant. CdSe fluorescence covers the visible light area of the spectrum, whereas CdTe uses the infrared sections. Since the QDs are hydrophobic, they must be functionalized with additional coatings or "capping" substances such mercaptopropionic acid and polyethylene glycol in order to increase their solubility and keep them from aggregating. These coatings may also be combined with molecules that direct the QD to a particular tissue or organ, such as receptor ligands or antibodies. QDs might thus significantly advance medical therapy in terms of cancer identification and treatment.

Early publications on the creation of intracellular semiconductor nanoppapers include E. When exposed to cadmium chloride and sodium sulphide, coli has been seen to collect nanocrystals made of wurtzite crystal with spherical and elliptical forms and sizes between 2 and 5 nm. When the E. is present, nanocrystal formation is said to be -fold greater. Compared to the late logarithmic phase, coli cells are grown to the stationary phase[8]. Additionally, it has been shown that spherical aggregates of 2–5 nm sphalerite ppapers develop in naturally

occurring biofilms dominated by aerobic sulfate-reducing bacteria of the Desulfobacteriaceae family. CdS, a semiconductor nanocrystal produced by C. thermoaceticum, E. coli, and Klebsiella pneumoniae. coli displayed elliptical and spherical forms in the 2-0 nm size range. From decreasing salt marsh sediments, Sharma et al. identified the extremely cadmium-resistant Klebsiella planticola strain Cd-1.

The strain was capable of growing in a broad range of NaCl concentrations and at neutral to acidic pH levels in up to mM CdCl2. According to X-ray absorption spectroscopy, the strain precipitates considerable quantities of cadmium sulphide in growth medium that have been supplemented with thiosulfate. When exposed to Cd2+ in the growth media, Klebsiella aerogenes produced spherical CdS crystallites that were attached to the cell wall as electron-dense ppapers in the range of -0 nm. According to energy dispersive X-ray analysis, cadmium and sulphur are present in a 1:1 ratio. By producing CdS nanocrystals extracellularly at ambient temperature, the purple, nonsulfur photosynthetic bacteria Rhodopseudomonas palustris was shown by Bai et al. The distribution of fcc-structured nanoppapers of size 8 was verified by TEM and electron diffraction investigations to be spherical. ± 0. size nm. The production of CdS nanocrystals has been attributed to cysteine desulfhydrase activity. The manufacture of -nm CdS nanoppapers has also utilised the bacterial cellulose obtained from the strain Gluconoacetobacter xylinus.

Nanoppapers of Selenium

Sulfurospirillum barnesii, B. subtilis, and other bacteria such as these use selenium oxyanions as the electron acceptor. Se nanoppapers measuring less than 0 nm are produced by selenitireducens, Selenihalanaerobacter shriftii, and other organisms in a consistent, stable, and crystalline form. Nanoppapers' spectrum characteristics are quite different from those of amorphous Se0 produced chemically by oxidising H Se and vitreous Se0 produced chemically by reducing selenite with ascorbate. The structure and spectral characteristics of selenium nanospheres generated by Se-respiring bacteria were described by Oremland et al. An isolated strain of Stenotrophomonas maltophilia SELTE, which transforms selenite to elemental selenium and accumulates selenium granules in either the cell cytoplasm or extracellular space, is a selenium hyperaccumulator legume called Astragalus bisulcatus. E. coli, a facultative anaerobic bacteria, is also present. cloacea SLD1a-1, the anoxic and oxic forms of the purple nonsulfur bacteria Rhodospirullum rubrum, and Desulfovibrio[9]

It has been shown that desulfuricans can bioreduce selenite to selenium both within and outside of the cell. E. Additionally, it has been shown that bacteria deposit elemental selenium in both the cytoplasm and periplasm. Selenite may also be aerobically reduced by P. stutzeri to elemental selenium. Yadav et al. recently shown that P. aeruginosa SNT1, isolated from rhizospheric seleniferous soil, produced nanostructured selenium by biotransforming selenium oxyanions both intracellularly and extracellularly to spherical amorphous allotrophic elemental red selenium. Selenium is used in photocopiers and microelectronic circuitry because of its photo-optical and semiconducting characteristics.

Nanoppapers of Titanium, Platinum, and Palladium

the filtrate from Lactobacillus sp. extracellular culture. has been shown to generate titanium nanoppapers at room temperature, forming spherical aggregates with sizes between and nm. Due to their reduced weight and resistance to corrosion, titanium dioxide nanoppapers are used extensively in a variety of products, including cars, cathode ray tubes, aeroplanes, submarines, and desalination plants. They also have a potential future in gene therapy and cancer treatment. TiO2 nanoppapers are suggested for usage as antibacterial agents, UV protectants, water and air purifiers, gas sensors, and high-efficiency solar cells since they also

display photocatalytic activity. Its structure, microstructure, and powder purity are all closely connected to its photoactivity. Anatase, rutile, and brookite are the three types of TiO2 crystals that are now recognised. Rutile has less photocatalytic activity than the anatase form of TiO2. In terms of thermodynamic stability, rutile is superior than brookite and anatase.

The extracellular platinum nanoppapers and metallic platinum nanoppapers with spherical, bead-like chains and dendritic morphologies in the ppaper size range of -0 nm have been reported to be produced by the gram-negative cyanobacterium P. boryanum UTEX 5. It has been shown that a stationary phase growth of the metal ion-reducing bacteria Shewanella algae in aqueous H2PtCl6 solution under anaerobic conditions at ambient temperature and neutral pH may convert PtCl 2 ions to metallic platinum in under a minute when lactate is present as an electron donor. Platinum nanoppapers of around 5 nm size have been shown deposited in the periplasmic region of the bacterial cell, which is located between the inner and outer membranes. D., a bacteria that reduces sulphate. In a matter of minutes at pH neutral, desulfuricans NCIMB anaerobically bioreduced and biocrystallized palladium ions to palladium nanoppapers on the cell surface[10].

The major source for the manufacture of secondary metabolites like antibiotics is often thought to be actinomycetes. However, there is still need for further research in the field of screening actinomycetes for their intrinsic capacity to synthesise nanoppapers. It has been shown that the extremophilic actinomycete Thermomonospora sp. produces extracellular monodispersed, spherical gold nanoppapers with an average size of 8 nm. The presence of amide and bands of protein as a capping and stabilising agent on the surface of nanoppapers was verified by Fourier transform infrared spectroscopy analysis. Additionally, the alkalotolerant actinomycete Rhodococcus sp. accumulated 5-nm gold nanoppapers within its cells. The cell wall's reductases decreased Au3+ and accumulated Au0 on the cytoplasmic membrane and cell wall.

CONCLUSION

The use of bacteria in the synthesis of nanocellulose materials, such as nanoppaper, offers a promising and sustainable alternative to traditional methods of material production. This microbial-based approach not only offers a more environmentally-friendly and cost-effective method of production, but also enables the customization of material properties through genetic manipulation and control of the culture conditions.

Nanoppaper synthesized by bacteria has unique properties, such as high mechanical strength, high porosity, and transparency, which make it suitable for a wide range of applications in various fields including packaging, electronics, and biomedical engineering.

However, the production of bacterial nanocellulose materials is still in its early stages and further research is needed to optimize the production process and improve the material properties. Additionally, the safety and regulatory aspects of bacterial nanocellulose materials need to be thoroughly investigated to ensure their suitability for various applications.

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CHAPTER 10

APPLICATIONS AND RANGE OF NANO PAPERS

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ABSTRACT:

It has been discovered that the cyanobacterium Plectonema boryanum UTEX 5 produces silver nano papers. Additionally, this filamentous cyanobacterium formed cubic gold nanopapers and octahedral gold platelets after being incubated with aqueous Au2 and AuCl solutions. The mechanism of gold bioaccumulation by cyanobacteria from a gold chloride solution suggested that its interaction with cyanobacteria promotes the precipitation of amorphous gold sulphide nano papers at the cell wall, and ultimately deposited metallic gold in the form of octahedral platelets near cell surfaces and in solution. Additionally, it has been shown that several common cyanobacteria, such as Anabaena, Calothrix, and Leptolyngbya, create intracellular Au, Ag, Pd, and Pt nanopapers. These papers are spontaneously discharged into the culture medium and are stabilised by algal polysaccharides enabling simple recovery. In fact, the cyanobacteria genus affects both the yield and the size of the retrieved papers.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

By generating a metal-thiolate combination with phytochelatins, the yeast Candida glubrata has been utilised to produce monodispersed, spherical, and peptide-bound CdS quantum dots measuring 2 nm within cells. Additionally, CdS nanopapers with wurtzite-like hexagonal lattice structures and sizes between 1 and 1.5 nm were generated by Schizosaccharomyces pombe in mid-log phase. It has been reported that the yeast Torulopsis sp. produced fcc-structured PbS nanocrystallites with quantum semiconductor characteristics. Quantum dots, which have a spherical form and a size of 2–5 nm, are created intracellularly in vacuoles. These nanopapers are utilised to create polymer-based diode heterojunction. Additionally, it has been noted that the aldehyde group found in reducing sugars causes Baker's yeast, Saccharomyces cerevisiae, to biosorb and convert Au3+ to elemental gold in the peptidoglycan layer of the cell wall. A different yeast called Pichia jadinii created 0 nm-sized spherical, triangular, and hexagonal gold nanopapers intracellularly in a matter of hours[1], [2].

Yarrowia lipolytica NCIM, a different tropical marine yeast, formed gold crystals with an average size of nm that were nucleated on the cell surfaces by the reduction of gold ions at pH 2.0. Additionally, it has been shown that S. cerevisiae can form spherical antimony oxide nanopapers with semiconductor characteristics in the 2-nm size range at room temperature. The likely mechanism might be membrane-bound quinine radial tautomerization or membrane-bound/cytosolic pH-dependent oxidoreductases. The exponential development phase of the silver-tolerant yeast strain MKY3 has also been shown to produce hexagonal silver nanopapers 2-5 nm in size extracellularly.

Fungi-based Nanoppaper Biosynthesis

Biosynthesis of metal nanopapers using fungi such as F. oxysporum , Colletotrichum sp. , Trichothecium sp., Trichoderma asperellum, T. viride, , Phaenerochaete chrysosporium , Fusarium solani USM , Fusarium semitectum , Aspergillus fumigatus , Coriolus versicolor , Aspergillus niger , Phoma glomerata , Penicillium brevicompactum , Cladosporium cladospori- oides , Penicillium fellutanum , and Volvariella volvacea has been extensively studied. In fact, fungi are preferred over other microorganisms for nanoppaper biosynthesis because their mycelial mesh is more resistant to flow pressure, agitation, and other conditions in bioreactors, they grow quickly and are manageable, they secrete more reductive proteins extracellularly, and they are simpler to process downstream. Additionally, the nanopapers that precipitated outside of the cell may be used directly to a variety of processes. The fact that nanopapers form inside the body may be connected to the size limit of such papers. These papers might be nanosized compared to nanopapers made extracellularly. Verticillium sp. was used by Mukherjee et al. to demonstrate the biological production of -nm gold nanopapers. On the cell walls of fungal mycelia, TEM study of ultrathin sections revealed primarily spherical shapes, a few triangles and hexagonal nanopapers, and quasi-hexagonal morphology. Additionally, it has been shown that Verticillium luteoalbum can form spherical -nm gold nanopapers in h at pH 3.0. At pH 5.0, however, spheres, rods, triangular, and hexagonal morphologies all developed. It has also been discovered that Trichothecium sp. intracellularly accumulates gold nanopapers [3], [4].

Additionally, after being exposed to an aqueous silver nitrate solution, Verticillium sp. Biomass accumulated silver nanopapers just below the surface of the fungal cell. It has been shown that Phoma PT and Phoma sp.3 preferentially collect silver nanopapers. According to Vigneshwaran et al., after being exposed to a silver nitrate solution for h, Aspergillus flavus accumulated silver nanopapers measuring 8.9 nm on the surface of its cell wall. One of the contributing elements to the substantially greater productivity of nanopapers in this biosynthetic technique might be the fact that fungus are known to produce far larger quantities of proteins than bacteria. Species-specific NADH-dependent reductase secreted by F. oxysporum has been employed to catalyse the reduction of AuCl4 ions to gold nanopapers in order to clarify the process of nanoppaper synthesis. Positively charged lysine residues in the mycelia cell wall may interact electrostatically with the surface of fungal cells to capture AuCl4 ions.

The aggregation of metal atoms might result from the reduction of the gold ions by cell wall enzymes; however, the precise method by which the gold nanopapers are formed is yet unclear. It has been proposed that proteins and other reducing agents released by the fungus stabilise extracellularly generated nanopapers. Experimental evidence points to the involvement of several high-molecular-weight proteins in the creation and stabilisation of nanopapers, notably the NADH-dependent reductase produced by fungal biomass. The native form of these proteins, which are present in solution and bonded to nanoppaper surfaces, is unaffected, according to fluorescence emission spectra, and the reduction of metal ions had no effect on the protein's tertiary structure.

The creation of nanopapers has been shown using proteins extracted from fungal cultures. For instance, silicatein-like cationic proteins released by F. oxysporum were capable of extracellularly hydrolyzing aqueous ZrF6 ions and producing nanocrystalline zirconia at room temperature. Growth circumstances are crucial for the production of nanopapers. When exposed to gold ions, stationary Trichothecium sp. biomass formed extracellular nanopapers. The fungus does, however, create intracellular gold nanopapers when disturbed. This may be due to the fact that under stationary circumstances, proteins and enzymes necessary for the

creation of nanopapers are released into the medium but under shaking conditions, they are not. F. oxysporum and Verticillium sp. were used to create magnetic nanopapers at room temperature, according to Bharde et al. Both types of fungus released the proteins needed to hydrolyze iron precursors and create iron oxides outside of cells. According to Bhainsa and D'Souza, A. fumigatus may produce monodispersed silver nanopapers in under a minute. Additionally, Bansal et al. showed how F. oxysporum can produce tetragonal barium titanate nanopapers with a size of nm under ambient circumstances. With their use in creating ultrasmall capacitors and ultrahigh density nonvolatile ferro- magnetic memory, these nanopapers' ferroelectric characteristics have the potential to completely transform the electronics industry. Furthermore, utilising the fungus F. oxysporum, Bansal et al. and Kumar et al. reported the production of highly luminous CdSe quantum dots, as well as silica and titania nanopapers [5], [6].

Applications and Range of Nanopapers

The creation of inorganic and metal-based nanomaterials has sparked the growth of a new sector that unites several scientific specialties in the search for various forms of nanopapers with distinctive features. In addition to being an intriguing topic of research, the design and development of new and economical methods for the scale-up manufacturing of nanomaterials will also answer the rising human needs, including health, safety, and environmental concerns. The usage of nanopapers in industry is spreading, and soon they will take the place of the poisonous or dangerous compounds now utilised as antibacterial agents. Utilising nanopapers and their nanocomposites provides a reliable and comparatively safer alternative, creating new possibilities for the creation of antimicrobials. Silver has been researched and utilised to combat illness and stop spoiling the most since ancient times. Compared to certain organic antimicrobial agents, it is a safer antimicrobial agent. Due to its bactericidal effects on items containing silver, mostly as a result of its antibacterial properties and minimal toxicity to human cells, silver has been referred to as being oligodynamic. Its therapeutic effectiveness has been shown against a variety of bacteria. Recently, Musarrat et al. showed that biosynthesized AgNPs had broad-spectrum antibacterial action against several human and plant pathogenic bacteria and fungi, including Citrobacter spp., Staphylococcus aureus, and Shigella dysenteriae type I[7], [8].

Similar to ZnO nanopapers and nanorods, these materials have impressive uses in photodiodes and UV light emitting devices, sunscreens, sensors, displays, gas sensors, piezoelectric devices, electroacoustic transducers, gas sensors, UV absorbers, antireflection coatings, photocatalysis, and chemical catalysts.Additionally, gold nanopapers are well recognised for their effective antibacterial properties against scurvy or acne and have practical uses in the soap and cosmetics sectors. They have the ability to regulate sebum and eliminate waste from the skin. Different Gram-positive and Gram-negative bacteria, fungi, and other microorganisms have had their growth inhibited by Au nanopapers, according to Zhang et al. The liposomes that Park et al. loaded with gold nanopapers might be employed as a delivery device with controlled release.

DISCUSSION

There are several uses for nanopapers in biology and medicine. They might be employed as probes connected to peptides, antibodies, or nucleic acids for the detection and measurement of chemical processes in vivo in a dynamic range of size 0 nm. Nanopapers are biocompatible for detection and medical diagnostics due to the possibility of covering them with antibodies, collagen, and other materials. According to Bruchez et al., fluorescent labelling using nanopapers is preferable than using regular fluorophores. According to Wu et al.,

immunofluorescent labelling of the cancer marker Her2 using quantum dots is more effective in labelling a variety of target cell-surface receptors, nuclear antigens, the cytoskeleton, and other intracellular organ- elles than using conventional fluorophores. Additionally, they showed how useful bioconjugated colloidal quantum dots were for in vivo imaging, cell tracking, DNA detection, and cell labelling. Zhang et al. demonstrated that ethylene glycol and folic acid surface modification of superparamagnetite nanopapers is efficient in promoting phagocytosis by cancer cells for future cancer treatment and detection. In mice, O'Neal et al. found that selective photothermal ablation of tumours using near-infrared-absorbing polyethylene-coated gold nanoshells of 0 nm size suppressed tumour development and improved animal survival for up to days in comparison to controls. Furthermore, the Perkel-reported antibody-coated magnetic iron nanopapers have been shown to heat and essentially burn tumours. According to Gopalan et al., a new tumour suppressor gene called FUSI may be used in nanoppaper-based gene therapy to treat lung cancer systemically. According to Dufes et al., gene therapy employing nanoppaper-based vector systems and a plasmid that expresses tumour necrosis factor led to enhanced transgene expression and prolonged rat life without any harm. Studies conducted in vitro on breast cancer cells have shown the effectiveness of delivering the wild-type p gene using nanopapers. When a gene was delivered to cancer cells using nanopapers, the antiproliferative activity in the cells amplified and persisted. For systemic delivery of tumor-targeted genes to solid tumours, Kaul and Amiji found that PEG-modified gelatin nanopapers were very effective, biocompatible, biodegradable, and long-circulating.

Another heavily researched subject in BioMEMS research is pathogen detection. Although they take hours to days to produce compliance, culture and colony counting techniques and PCR have been the two traditional and most selective/reliable approaches in molecular biology labs. The focus of detecting technologies has shifted to BioMEMS/sensor technology because it delivers just as dependable results in a quarter of the time as traditional approaches.

For the production of metal nanopapers during the last several years, a number of techniques based on chemical reduction, thermal treatment, irradiation, laser ablation, etc. have been utilised. The majority of these techniques significantly depend on the employment of hazardous reducing agents such sodium borohydride and N, N-dimethylformamide as well as organic solvents, which may cause serious environmental issues as well as biological dangers. The potential of bacteria, fungi, and even plants for the synthesis of nanopapers as eco-friendly nanofactories has led to the rise of biological and biomimetic techniques for green synthesis of nanomaterials. According to reports, oxidoreductases and a shuttle quinone extracellular procedure have been used to reduce metal ions to nanopapers using the cell mass and leached components from microorganisms.

Due to their simplicity in handling, ability to be mass-cultivated, high metal tolerance, capability to adhere to walls, and potential for intracellular metal absorption, filamentous fungus have several specific benefits over bacteria. Due to their distinct chemical and physical characteristics that set them apart from the corresponding bulk material, nanopapers of noble metals like gold, platinum, palladium, and silver, among others, have caught the interest of scientists in recent years. They are valuable for numerous applications, including nonlinear optics, spectrally selective coatings for solar energy absorption, optical receptors, catalysis in chemical processes, biolabelling, and as antibacterials, because to their exceptionally tiny size and enormous surface area in comparison to their volume. Therefore, using biologically compatible materials for the synthesis and stabilisation of nanopapers could be extremely important for medical diagnosis and therapeutics, such as the color-coded

fluorescent labelling of cells for the detection of genetic disorders using semiconductor quantum dots, as well as cell transfection for gene therapy and drug delivery.

Quorum sensing is the technique by which bacteria gauge the density of their population by speaking to low-molecular-weight ligands. Both Gram-negative and Gram-positive bacteria have several quorum sensing mechanisms. Acyl homoserine lactones are the signal molecules that Gram-negative bacteria employ the most often. Bacterial QS has been the subject of a significant quantity of paper and data recently. Scientific groups now show more interest in modulating quorum sensing using various techniques. In this chapter, we update our description of bacterial quorum sensing and discuss potential strategies to prevent AHL-based quorum sensing as well as assays and techniques for identifying signal molecules. This paper discusses the importance of QS interference caused by prokaryotic and eukaryotic species in connection to plant health and the environment.

Tomasz revealed the first instance of cooperative behaviour among microorganisms over a decade ago. Nealson et al. used a cell-density-dependent reaction to study the biology of light-producing organelles in deep-sea fish using the bacterium Vibrio fischeri. Fuqua et al. later used the term "quorum-sensing" to describe this population-dependent phenomenon, reflecting the minimal threshold of individual cell mass necessary to launch a coordinated population response. A low-molecular-mass signalling molecule, whose extracellular concentration rises with the population density of the generating organism, is produced and exported intracellularly to coordinate such a system. Due to their origin within the bacterial cell and ability to control their own expression, the signal molecules engaged in this communication are known as "autoinducers." Once a critical concentration or "quorum," i.e., the minimum number of bacteria amassed within a volume to make the "decision" to switch on gene expression of QS-controlled genes, is reached, the signalling molecule can be sensed and reimported into these cells, allowing the entire population to respond to changing environment/requirements[6], [9].

The most well-studied QS systems in Gram-negative bacteria utilise N-acyl homoserine lactones produced by LuxI-type enzymes as signal molecules to bind and activate members of the LuxR transcriptional activator protein family.One of the quorum signalling molecules for V. fischeri has been identified as the autoinducer N--N homoserine lactone. By catalysing the reaction between S-adenosylmethionine and acylated-acyl carrier proteins to create 3-oxo-C6-HSL, the LuxI autoinducer synthase produces this chemical. The luxI gene is located in the right-hand section of the bidirectional lux operon, which also contains the genes for the proteins involved in bioluminescence in addition to luxI. The left operon encodes the luxR gene, which is the 3-oxo-C6-HSL-dependent response regulator.

Bacterial Quorum Sensing Pathways

In both Gram-negative and Gram-positive bacteria, a surprising variety of signalling molecules serve as regional sensors to communicate population densities. Up to four QS systems may be used to broadly categorise these chemical signals and their receptors.The luxR/luxI autoinducer type 1 system in Vibrio spp., which was initially discovered of V. fischeri, is largely comparable to the autoinducer type 1 system that is commonly utilised in other genera of Gram-negative bacteria. A sequence of N-acyl homoserine lactone molecules are signalled by a highly soluble and readily diffusible sensor molecule. The length of the N-acyl chain, level of saturation, and number of oxygen substitutions in AHL molecules varies. All AHLs share the homoserine lactone ring in its L-isomeric form. The regions that facilitate the production of the AHL signalling molecule are encoded by the luxI gene or one of its homologs.

The luxR gene in V. fischeri or homologous genes in other bacterial species mediate the receptor for AHL. The LuxR receptor molecule is the translated form of luxR, and it works as a coactivator complex at the promoter sites of QS sensitive operons in the bacterial genome together with its AHL partner. The ultimate consequence in V. fischeri is bioluminescence, but for certain bacterial pathogens, the outcome is the activation of several virulence factors.There may be some interspecies communication since several bacterial genera share the same AHL molecule. other microorganisms, such as

System for Autoinducer Type 2 Signalling

Initially found in the V. harveyi bioluminescence system, the second QS route is mediated by the luxS gene locus and similar homologues. Nearly 50% of all sequenced bacterial genomes have evidence of the autoinducer type 2 system, which is now known to be the most common signalling mechanism used by both Gram-negative and Gram-positive bacteria. The AI-2 pathway enables effective bacterial signalling by using a more intricate, two-component receptor kinase network. The AI-2 signal in Vibrio species is structurally made up of complex, multiple-ringed, cyclical furanosyl molecules that feature the very uncommon presence of a boron atom[10].

With a number of gene products serving as the receptor kinase signal transcription complex, the receptor for the AI-2 apparatus is likewise intricate. The receptor is a membrane-bound, two-domain sensor kinase and response regulator in Vibrio species. In enteric bacteria, a soluble receptor attaches to the AI-2 signal molecule in the periplasmic space and then uses a particular ABC-type transporter system to move the AI-2 molecule across the membrane. An intracellular receptor that serves as the transcriptional activator complexes with the internalised AI-2 molecule after it has been phos- phorylated.

Bacteria have several versions of the AI-2 system. In Salmonella spp., the AI-2 signalling molecule is a boron-free furan molecule. Numerous bacteria reportedly express the AI-2 receptor complex instead of the luxS gene. Some bacterial strains may be able to detect and utilise AI-2 signals produced by other bacteria in this way to control their own coordinated transcriptional responses. Since most of the transcriptional activity of AI-2 systems is focused on the control of metabolic pathways, its exact function in bacterial pathogenicity is unclear.

System for Autoinducer Type 3

Perhaps the most complex signalling route to date has been the recently discovered QS system. The autoinducer type 3 system is similar to the AI-2 system in that it activates the virulome's genes using a two-component receptor kinase intracellular signalling complex; however, unlike AI-2, the AI-3 system can also be signalled by the human stress hormones epinephrine or norepinephrine.

Recently, the QseBC complex, the periplasmic receptor for the AI-3 system, was characterised. The sensor kinase QseC and the phosphorylated response regulator QseB are responsible for changing the transcription of the virulence genes. The pathogenesis of enterohemorrhagic E. coli infections and shigellosis depends on the AI-3 system. Enteropathogenic E. coli strains, commensal E. coli strains, and a variety of other Gram-negative, enteric species have all shown evidence of the AI-3 signalling network's components, whereas Gram-positive bacteria have not. Quorum-sensing systems are also known to be used by a variety of Gram-positive bacteria. When compared to the capabilities and bacteriocin production of Gram-negative organisms, the nature of the signal molecules utilised in these systems is different. The majority of autoinducing peptide signals are produced by cleaving bigger precursor peptides, followed by changes such isoprenyl group

replacement, formation of lactone and thiolactone rings, and lanthionines. Dedicated oligopeptide exporters are required for signal release from the cell, while sensor histidine kinases found in the cytoplasmic membrane are responsible for signal perception.

Numerous quorum-sensing signals and various peptides are used by many Gram-positive bacteria to interact. In rare instances, the signalling peptide may be recognised by various strains of the same or similar species in addition to its cognate species.

CONCLUSION

Nanocellulose materials, including nanoppaper, offer a wide range of applications due to their unique properties such as high mechanical strength, biodegradability, and sustainability. Nanopapers have the potential to replace traditional paper products and synthetic materials in various fields including packaging, electronics, and biomedical engineering.The applications of nanopapers are still being explored, but they have already shown promising results in areas such as water filtration, wound dressing, and drug delivery.

The use of nanopapers in electronics is also gaining attention due to their excellent mechanical and electrical properties. However, there are still challenges that need to be addressed to further expand the applications of nanopapers, including the need to improve the scalability of production, address safety concerns, and optimize the material properties for specific applications.

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CHAPTER 11

A STUDY ON QS SIGNAL MOLECULES DIVERSITY

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ABSTRACT:

Quorum sensing (QS) is a cell-to-cell communication system used by many bacteria to coordinate their behavior in response to changes in cell density. QS signal molecules are small diffusible compounds that mediate intercellular communication, enabling bacteria to regulate various physiological processes, including virulence, biofilm formation, and antibiotic resistance. In recent years, the diversity of QS signal molecules has been increasingly recognized, with a growing number of novel compounds identified from a wide range of bacterial species. This review provides an overview of the diversity of QS signal molecules, including their chemical structures, biosynthesis, and functions. The review also discusses the ecological and evolutionary significance of QS diversity, highlighting the potential for QS-based communication to enable bacterial adaptation to different environments and facilitate interspecies interactions. Moreover, the review discusses the challenges and future prospects of studying QS signal molecules diversity, emphasizing the need for improved methods for their detection, characterization, and functional analysis.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

The majority of QS signals are either tiny chemical compounds or 5-amino-acid peptides. For instance, N-acyl homoserine lactones, 2-alkyl-4-quinolones, long-chain fatty acids, fatty acid methyl esters, as well as autoinducer-2, a name for a collection of interconvertible furanones generated from dihydroxypentanedione, are used by gram-negative bacteria. Although usually speaking these organisms prefer linear, modified, or cyclic peptides such as the autoinducing peptides generated by the staphylococci, certain Gram-positive bacteria also make AI-2. However, the streptomycetes produce c-butyrolactones like A-factor, which structurally resemble AHLs since both chemical types are butanolides. Generally speaking, however, Gram-positive bacteria use autoinducing peptides, which vary in length from 5 to amino acids and often have peculiar chemical structures, as quorum-sensing signal molecules. Three distinct families of AIPs have been identified based on their distinct structural characteristics: the oligopeptide lantibiotics, typified by the lactococcal nisin, which are distinguished by the presence of lanthionine-mediated thioether macrocyclic features and dehydroamino acid residues; the -membered thiolactone peptides, exemplified by the staphylococcal AIP-1; and the is Additionally, QS signal molecules may be split into groups based on whether they internalise or interact with cell surface receptors. But in this paper, we focus mostly on quorum-sensing signal molecules and how Gram-negative bacteria might block them[1].

Numerous QS systems in bacteria have been reported since QS regulation was first discovered in V. fischeri. They control a variety of processes, including the synthesis of antifungal or antibiotic substances, motility patterns, virulence factors, biofilm development, and plasmid conjugation transmission. P. aeruginosa's las and rhl quorum-sensing systems control at least 6% of its genome via AHL. Together with LasR, LasI controls the production of virulence factors such as elastase, LasA protease, alkaline protease, and exotoxin A. LasI largely controls the synthesis of N--L-homoserine lactone. RhlI controls the creation of C4-HSL, which activates RhlR and causes the production of rhamnolipids, elastase, LasA protease, hydrogen cyanide, pyocyanin, siderophores, and LecA and LecB lectins, among other things.

The violacein and chitinase-inducing N-hexanoyl-L-homoserine lactone enzyme is encoded by the cviI gene in Chromobacterium violaceum. In C. Burkholderia cepacia, also known as cepR and cepI, has been shown to produce a variety of phenotypes that are known to be QS-regulated luxRI homologues, including violacein, hydrogen cyanide, and exoprotease. Both protective and restrictive regulatory functions are performed by the CepRI quorum-sensing system in B. Cepacia produces more proteases while generating fewer siderophore molecules. E. Carotovora successfully destroys plant tissue while avoiding plant defences by using quorum sensing for exoenzyme synthesis. Exoenzymes ExpR and ExpI, homologues of LuxI, control the synthesis of these exoenzymes. E. The discovery that carbapenem synthesis is controlled by a second quorum-sensing mechanism makes carotovora quorum sensing considerably more complicated. CarR and CarI, the latter of which catalyses the synthesis of 3-oxo-C6-HSL, control the generation of carbapenems[2].

A. As a result, it is likely that conjugal transfer occurred in A. tumefaciens. This is because the organism generates 3-oxo-C8-HSL, which induces plasmid conjugation together with a regulator called TraR and may activate production of the tra genes. A quorum-sensing mechanism controls tumefaciens. Additional pathogens, including P. aeruginosa, A. along with S. Marcescens has been shown to control the development of biofilms using QS. Numerous bacterial species have been found to generate AHL signals, but the underlying biological mechanism is still unknown. A number of reports have

Purification, Characterization, and Isolation of AHL Molecules

Late-exponential phase cultures' wasted supernatants may be used to extract AHLs. In a nutshell, microorganisms are eliminated using centrifugation. Then, supernatants are twice extracted with equal volumes of ethyl acetate, and the extracts are dried over anhydrous magnesium sulphate, filtered, and evaporated to dryness. Khan et al. isolated AHL from Chromobacterium violaceum 2 by modifying the conventional technique. A 4 L culture of bacteria is cultured in a shaking incubator at °C for h, then centrifuged at,0 g. Membrane filteration sterilises the supernatant.

The filtrate is extracted using acidified ethyl acetate before being concentrated, dried, and reconstituted in acetonitrile using rotary evaporation.

Very low quantities of QS signal molecules are created in the presence of numerous interfering substances. To date, several qualitative and quantitative techniques have been developed to detect AHLs. The majority of these detection methods concentrate on the presence of diverse AHLs in different bacterial cultures to establish their stimulating effects in quorum-sensing mechanisms. Whole-cell-based bioassays using AHL-specific biosensors, thin-layer chromatography, gas chromatography-mass spectrometry, high-performance liquid chromatography, liquid chromatography-mass spectrometry, isotopic labelling, and absorbance-based assays are a few of these[3].

Identification Using Bioassays

The development of several bioassay strains has made it easier to identify acyl HSLs in microbiological samples and has led to the creation of numerous bioassays and sensor systems that make microbial acyl HSL detection, characterisation, and quantification simple. These strains lack all AHL synthases and have a readily assayable reporter gene, therefore exogenous AHLs are necessary for reporter activity. The use of bacterial biosensors that can identify the presence of AHLs has been crucial in enabling the identification of the AHL system to date. The functioning LuxR family protein in the biosensors, together with a corresponding target promoter, are cloned together and positively control the transcription of a reporter gene even when the biosensors don't make AHLs. AHL biosensors have been developed based on multiple LuxR family proteins that can recognise AHLs with short, medium, and long acyl chains as well as 3-hydroxy-AHLs. The generation of an endogenous pigment as well as the reporter genes lacZ, gfp, lux, and others have all been used. In agar-plate-based bioassays, many reporter strains have been used, although all techniques are essentially the same. The test strain or extract is then spotted on top of the agar or in wells after the reporter strain and agar have been combined. The vicinity of the spots or wells are examined for the presence of the reporter gene product after an adequate amount of incubation. This method will not, however, reveal if a sample has more than one signal molecule or just one. But detection may be carried out via thin-layer chromatography.

AHL biosensors substantially assist the analysis of the quorum-sensing signal molecule generated by a specific organism by monitoring using T-streaks or tests of conditioned medium. Due to bacteriostatic or bactericidal effects on the biosensor, other substances generated by the target organism might provide false-negative findings in these experiments. This issue may be resolved and any AHL present can be concentrated by removing AHLs from used growth media using organic solvents. When feasible, target organisms may be transformed using biosensor platforms to avoid the issue with antimicrobial activity. Furthermore, any cell-density-dependent production may be determined using the test of reporter gene expression during growth in these altered strains. The ideal vectors for this kind of investigation are broad-host-range vectors like pSB3, which is based on pRK5. It has also been successful to find E recombinant clones of the AHL synthase gene using AHL biosensors. coli. An E may be introduced using genomic libraries created from species triggering the biosensor. It is possible to test for reporter activation using an E. coli strain that has an AHL reporter plasmid and the transformants that come from it. As an alternative, biosensor overlays may be used to screen altered libraries[4].

Analytical Chemistry

Methods often used to analyse autoinducers include thin-layer chromatography, gas chromatography, high-performance liquid chromatography, and isotope labelling. TLC combined with a particular bioassay is an efficient method that enables the testing of a wide range of isolates. It helps with autoinducer detection, measurement, and purification monitoring. By fractionating concentrated supernatant extracts using HPLC or TLC, AHLs may be made pure. On the basis of variations in mass and polarity, organic molecules in the supernatant are separated.

TLC offers a quick and easy method for determining the minimum amount of various acyl-HSL species generated by a certain organism when combined with a bioreporter. Fractionation and detection may be accomplished in h using TLC overlay methods. On the basis of Rf, spots of pigmentation or bioluminescence may be photographed and compared to recognised standards. The method also provide a limited amount of preliminary information

on the nature of these complaints pounds found in culture supernatants extracts. Rf values derived for the samples may be compared with those of the standards. TLC also offers preliminary information on the quantity and kind of compounds present in the supernatant of bacterial cultures. C reversed-phase TLC plates may be used for residual separation. However, it is possible to assign structures based on spectroscopic characteristics rather than only chromatographic ones. Infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy are the most useful instruments for characterising substances[5].

For the fractionation and isolation of AHLs for structural study, HPLC is a successful technique. Active HPLC fractions may be found using biosensors, and these fractions can then be submitted to nuclear magnetic resonance spectroscopy and mass spectrometry. Chemical synthesis may then be used to confirm the anticipated molecule's structure. A modified version of the colorimetric test published by Goddu et al. for the analysis of ester molecules was created by Yang et al. This approach can analyse the number of lactone compounds and lactonase activity and is quick and simple to apply. Comparable to the HPLC approach, its detection limit for lactone molecules is around 1 nmol.

Use of Chemical and Microbiological Assays

McClean et al. created Chromobacterium violaceum, a biosensor-using organism that makes and reacts to C6-AHL. C. A transposon is introduced into the cviI AHL synthase gene and another into a potential locus for the violacein repressor in the violaceum CVO strain, a violaceinand AHL-negative double mini Tn5 mutant. When the CVO strain is exposed to exogenous AHL, violacein is produced quickly. The natural agonist AHL for CVO is the most effective. C. the C6-AHL, or violaceum AHL. C6-3-oxo-AHL, C8-AHL, C8-3-oxo-AHL, and C4-AHL are the additional AHLs that induce reasonably. C4-3-oxo-AHL has relatively little effect on the strain, while AHLs with acyl chains longer than C have no effect. Furthermore, CVO does not always identify 3-hydroxy-AHLs. This strain is suitable for detection on solid medium using both the TLC soft agar overlay method and "T" streak analysis.

With P. aeruginosa PAO1 MLZ, a lasI genomic knockout mutant that possesses a transcriptional fusion of promoter of rsaL and reporter gene lacZ, long-chain AHLs, in particular C-3-oxo-AHL, may be detected and quantified. The LasI/R AHL QS system directly controls the expression of the rsaL gene. When C-3- oxo-AHL is given to PAO1 MLZ, this causes rsaL transcription through LasR, which can be measured by looking at -galactosidase activity. Additionally, this sensor is used for C-3-oxo-AHL.

The Agrobacterium tumefaciens-based AHL biosensor has the highest sensitivity to these substances and can detect a variety of AHLs. The three-plasmid system in a makes up the biosensor. tumefaciens KYC, a strain that doesn't have a Ti plasmid and doesn't make AHL. The phage T7 promoter controls the TraR gene in plasmid pJZ4; the RNA polymerase gene is found in pJZ0; and the traI-lacZ reporter fusion is found in pJZ2. This biosensor is very sensitive to a variety of AHLs and is capable of detecting very low concentrations. Similar to another A. It may also be used to TLC analysis when utilised with tumefaciens-based biosensors. This biosensor strain may also be used to directly assess extracts or supernatants and determine quantification. The Ti-plasmid contains the well-characterized TraI/R AHL system, which generates and reacts to C8-3-oxo-AHL.

Biosensor A for AHL. tumefaciens NT1 is a strain of NT1 that has had its Ti plasmid removed, rendering it unable of producing AHLs. The traR gene is located on the plasmid, and the Ti plasmid conjugal transfer is carried out by one of the tra operons that includes a traG-lacZ reporter fusion and whose transcription is controlled by the TraI/R AHL QS

system. It needs relatively modest quantities of AHL extracts since it is sensitive enough to several AHLs. When using the sensor, colonies, supernatants, or sample extracts are spotted onto a sensor overlay that has been grown in an appropriate medium that contains X-gal. The presence of AHLs causes a blue zone to form around the application site after an overnight incubation. identical A. plasmid pCF8, which robustly produces the AHL-responsive transcriptional factor TraR, and a plasmid pCF2 bearing a TraR-regulated promoter traI transcriptionally linked to lacZ, were utilised to build tumefaciens biosensors. This A. AHLs with C6-C AHL chains are extremely sensitive to the tumefaciens biosensor. Blue colouring in the biosensor caused by lacZ expression and X-gal hydrolysis served as a sign that the AHL production test was successful[6].

Other biosensors have been created that rely on plasmid constructs that carry the Photorhabdus luminescens luxCDABE operon, which produces bioluminescence as a reporter system. Typically, Escherichia coli, which doesn't make AHLs, is home to these plasmids. Both the plasmids pSB1 and pHV0I are based on the V. fischeri LuxR and related luxI promoter that govern luxCDABE expression. As a result, the presence of AHLs causes bioluminescence, which may be readily recognised in a TLC analysis by exposing the TLC overlay with a biosensor to autoradiographic paper.

They have high sensitivity for cognate C6-AHL, C8-3-oxo-AHL, and C8-AHL and are most sensitive to cognate C6-3-oxo-AHL. These biosensors are difficult to utilise since they need photon camera equipment. A pair of LuxR biosensors, E. E. coli and C. coli, may be used in conjunction with a luminometer to quantify AHLs. The benefit of being able to be harboured in several additional Gram-negative bacteria is provided by the fact that pSB3 comprises the identical arrangement of pSB1 cloned in a wide-host-range mobilizable platform. E is a biosensor capable of detecting C4-AHL. coli . The ahyR gene from Aeromonas hydrophila and the associated ahyI gene promoter linked to luxCDABE are utilised to create the plasmid. Likewise, E. Another plasmid-based sensor that responds to C4-AHL is coli. This construct consists of luxCDABE coupled to the homologous promoter rhlI and rhlR. C4-AHL is the appropriate signal molecule for Pseudomonas aeruginosa's RhlI/R AHL QS system. The ideal location for this plasmid-based sensor is inside

E. mutant coli sdiA gene. E contains the orphan LuxR family protein SdiA. coli that may make the rhlI promoter active, hindering the ability to detect C4p-AHL. E. Coli does not produce AHL because it lacks a LuxI family synthase that is SdiA cognate. The lasR gene and cognate lasI gene promoter regulating the production of luxCDABE are found in the plasmid sensor pSB. You may har- bore this plasmid in E. C-oxo-AHL, C-oxo-AHL, and C-AHL are effective against E. coli.

The LuxI/R system is also the foundation for the pKDT coli plasmid sensor. This plasmid has a lasB-lacZ translational fusion and lasR that is controlled by the lac promoter; as a result, the response to exogenous AHL is determined by the activity of -galactosidase. The LasI/R AHL QS system regulates an elastase that is encoded for by the lasB gene. The E. The coli AHL biosensor is highly responsive to C-AHL, C-AHL, and their derivatives; however, it is unable to detect any of the shorter or 3-hydroxy AHLs. AHL may be measured using any of the two biosensors[7].

Specific sensors have been created based on the PhzI/R AHL QS system of Pseudomonas flourescens 2 to detect 3-hydroxy-AHLs. PhzI/R controls the expression of the phzABCDEFG operon, which produces the antibacterial chemical phenazine-1-carboxylate. The main and cognate signal of the six distinct AHLs produced by PhzI of P. fluorescens is C6-3-hydroxy-AHL. The two plasmid systems that make up the biosensor are found in the

wild-type P. fluorescens strain, which doesn't make AHLs. The phzR gene is located on plasmid pSF5 and is regulated by the trc promoter. The pSF7 plasmid houses the divergent phzR-phzA gene.

DISCUSSION

uidA and lacZ reporters, which are visible via -glucuronidase and -galactosidase activity, are coupled with a PhzR-regulated dual promoter region. The AHL sensor reacts best to C6-3-hydroxy-AHL and is ten times less sensitive to C8-3-hydroxy-AHL when using the phzA-lacZ reporter in pSF7. -glucuronidase or -galactosidase activity measurements may be utilised to quantify samples using this sensor in TLC analysis. Through the activation of lasB-lacZ transcriptional fusion, a new plasmid has been created and introduced into Pseudomonas aeruginosa QSC5 to enable the detection of a wide variety of acyl-homoserine lactones. Using the Vibrio harveyi strain BB0-autoinducer bioassay to identify the quorum-sensing autoinducer-2 molecule in culture fluids of commensal intestinal bacteria, it was demonstrated that P. aeruginosa could detect the presence of eight acyl-homoserine lactones tested at physiological concentrations. AI-2-like compounds were found in the culture fluids of Ruminococcus albus, Ruminococcus flavefaciens, Roseburia intestinalis, Eubacterium rectale, Clostridium proteo- clasticum, Bacteroides vulgatus, and Lachnospira multipara. Several authors have recently reported using several biosensor strains to detect signal chemicals.

Bacterial Quorum Sensing Interference

Inhibiting quorum sensing simply prevents the desired phenotype since quorum sensing is not necessary for bacterial survival and only aids in the coordination of community-based bacterial behaviour. For instance, inhibiting QS may diminish biofilm development, lessen bacterial virulence, and enhance bacterial susceptibility to medication treatment. In other situations, activating quorum sensing at an early stage may also lessen bacterial virulence. Therefore, it's possible that inhibiting quorum sensing in bacteria won't have a generally positive impact. Additionally, because no necessary bactericidal and/or bacteriostatic effects are produced, inhibition is important in the manufacture of adjuvants. Furthermore, at least six different QS routes have been discovered so far, proving that bacterial quorum sensing is not a one-time occurrence[8].

For example, only Gram-negative bacteria employ acylated homoserine lactones as autoinducers, while only Gram-positive bacteria use autoinducing peptides as autoinducers. distinct bacteria may use distinct routes and autoinducers to control QS. However, quorum sensing in both Gram-positive and Gram-negative bacteria is mediated by autoinducer-2. Scientists have discovered many ways to manage illnesses in both people and plants, however, by suppressing QS. However, suppressing QS should provide researchers additional information to investigate the precise role that these systems play in bacterial cell-cell communication, as well as the characteristics and genes involved in this process.

Many researchers have reported various methods for inhibiting quorum sensing in each pathway, including:

1. preventing the production of signal molecules
2. A signal transduction blocker inhibition of autoinducer secretion/transport
1. Targets are inhibited after receptor binding
2. Using, for instance, antibodies against autoinducers, sequestration of autoinducers
3. Signal molecules' biodegradation and chemical inactivation autoinducer degradation using enzymes or catalytic antibodies

4. Molecular inhibition of receptors using antibodies to "cover" autoinducer receptors, so blocking them.

Antagonism of Autoinducer Receptors

However, our emphasis in this chapter is on inhibition of AHL-mediated QS. These techniques may be used to accomplish inhibition in AIP-mediated QS, AHL-mediated QS, and AIP-2-mediated QS.

Blocking AHL-Mediated QS

Blocking the Biosynthesis of Signal Molecules

Different bacteria may share a single AHL or use separate autoinducers, although most Gram-negative bacteria utilise AHLs as signalling molecules in quorum sensing. The development of quorum-sensing inhibitor compounds that specifically target AHL signal production may take use of knowledge regarding signal creation. It has been shown that a number of SAM analogues, including S-adenosylhomocysteine, S-adenosylcysteine, and sinefungin, are effective inhibitors of AHL production, which is catalyzed. Protein RhlI from P. aeruginosa. The crucial initial step in the AHL-mediated quorum-sensing pathway is the synthesis of AHL compounds by a LuxI or its homologue. Since S-adenosylmethionine serves as the precursor for AHL, quorum sensing mediated by AHL may be disrupted by inhibitors of SAM-utilizing enzymes.

According to Parsek et al., S-adenosylmethionine analogues significantly reduced the activity of RhlI, the Pseudomonas aeruginosa LuxI homologue. Since no AHL synthase sequence patterns were discovered in other enzymes with S-adenosylmethionine binding sites, the S-adenosylmethionine analogues may be used as precise quorum-sensing inhibitors in prokaryotic or eukaryotic organisms without interfering with other crucial functions. Halogenated AA analogues were shown by Lesic et al. to particularly inhibit HAQ production and impair MvfR-dependent gene expression.

These substances decreased osmoprotection, a widespread bacterial function, and limited P. aeruginosa systemic dissemination and death in mice without affecting bacterial survival. The extracellular signals 4-hydroxy-2-heptylquinoline and 3,4-dihydroxy-2-heptylquinoline, whose synthesis depends on anthranilic acid, the primary precursor of 4-hydroxy-2-alkylquinolines, are what activate the Pseudomonas aeruginosa MvfR-dependent QS regulatory pathway.

This pathway regulates the expression of key virulence genes. These substances serve as a foundation for the design and development of selective anti-infectives that impede the pathogenesis of human P. aeruginosa and maybe other therapeutically important infections.

Signal Transduction Blocking

An antagonist molecule that is able to compete with or obstruct the native AHL signal's ability to attach to the LuxR-type receptor may prevent quorum-sensing signal transduction. Competitive inhibitors could have structural similarities with the natural AHL signal in order to attach to it and take up residence in the AHL-binding site while failing to activate the LuxR-type receptor.

Noncompetitive inhibitors, which bind to several locations on the receptor protein, may have little to no structural resemblance to AHL signals. In vitro applications of AHL analogues to block the quorum-sensing circuits of different bacteria are described in many papers.

Synthetic Autoinducer Analogues for Quorum Sensing

The effects of several structural analogues for various AHL molecules on the quorum-sensing mechanism of the associated bacterial strain have been investigated. The type of the agonistic or antagonistic action greatly relies on the expression of the TraR protein, according to a research on the analogues of 3-oxo-C8-HSL. Most AHL analogues are recognised as agonists when the response regulator is overexpressed, as is the case with numerous reporter strains. In the Gonzalez and Keshavan investigation, 3-oxo-C7-HSL, 3-oxo-C-HSL, and 3-oxo-C-HSL were all identified as agonists by wild-type levels of TraR. AHL-dependent gene expression was largely suppressed by the substances examined. As far as antagonists go, C8-HSL, 3-oxo-C6-HSL, C7-HSL, C-HSL, and 3-OH-C9-HSL were shown to be the most potent. When TraR was overexpressed, the bacterium was more sensitive to low concentrations of 3-oxo-C8-HSL than its parent strain. Overall, the agonistic or antagonistic activity, each requiring binding to TraR, is effective only when the acyl chain lengths are closer to that of the cognate AHL, 3-oxo-C8-HSL.

The homoserine lactone ring is crucial for biological activity, according to investigations of homologous AHLs of different quorum-sensing systems, but the structure of the acyl chains was not crucial for binding the response regulator. Based on the theory that altering the conserved lactone head group of AHLs would likely produce antagonistic compounds with a wider variety of applications, Olsen et al. created AHL analogues. Based on their results, they came to the conclusion that C-4 substitutions on the lactone ring resulted in weak activators of LuxR, suggesting that this region of the molecule is essential for recognition by LuxR, while replacement at the third position on the lactone ring produced activators of LuxR.

Although they seemed to be less effective than a furanone, two compounds containing carbamate lactones were recognised as inhibitors. Another research focused on creating 3-oxo-C6-HSL and C6-HSL analogues by adding ramified cycloalkyl or ramified aryl substituents to the acyl chain's C-4 position. The authors came to the conclusion that if one branch is added at the C-5 location of the acyl chain, the inducing activity is conserved. Compounds having a phenyl group or a phenyl carrying a heteroatom in the para position made the best antagonists. Because of steric hindrance, naphthyl and biphenyl compounds did not exhibit any action. Additionally, they noticed that phenyl derivatives' antagonistic action is favoured by the 3-oxo group, which is crucial for the inducing activity. Overall, when tested with an E, the secondary alkyl derivatives exhibited agonistic action whereas the aryl and tertiary alkyl derivatives exhibited antagonistic activity. coli-based luminous biosensor strain that has a plasmid with V. fischeri lux genes.

An equivalent of the cholera-causing bacteria Vibrio cholerae's CAI-1 autoinducer was created by Kelly et al. Response to two external quorum-sensing molecules, or autoinducers, regulates the production of virulence factors and the growth of biofilms. -3-hydroxytridecan-4-one, also known as CAI-1, was previously shown to be the strongest autoinducer. CqsA is necessary for CAI-1's biosynthesis. These researchers established the CqsA reaction mechanism, identified the substrates as 2-aminobutyrate and decanoyl coenzyme A, and showed that the process's end product, 3-aminotridecan-4-one, also known as amino-CAI-1, is the result of the reaction. A pyridoxal phosphate-dependent acyl-CoA transferase process caused CqsA to generate amino-CAI-1. In a later phase, amino-CAI-1 is transformed into CAI-1 via a CqsA-independent process. They also discovered that cells release CAI-1 0 times less often than amino-CAI-1. However, although other CAI-1 variants do not trigger a quorum-sensing response in V. cholerae, it does in amino-CAI-1. As a result, CAI-1 and amino-CAI-1 both have the potential to serve as lead molecules in the creation of an anti-cholera therapy.

Chan et al. have discovered the aiiA homologue of the bacterial strain KM1S, obtained from a soil sample from a Malaysian rainforest, which encodes an autoinducer inactivation enzyme that catalyses the destruction of N-acylhomoserine lactones. It was possible to select quorum-quenching bacteria, which were then amplified and cloned, by using a prescribed enrichment medium. According to sequence research, N-acylhomoserine lactone lactonases include the pattern 6HXDH- amino acids-H9- amino acids-D1. In vitro, it broke down the homoserine lactones N-3-oxo-hexanoyl and N-3-oxo-octanoyl.

The structure-function connections of AHL signals have been extensively studied in these research, and this information is very valuable for the ongoing quest for effective quorum-sensing inhibitors. These AHL antagonists may be produced synthetically and are categorised according to whether the lactone ring, the acyl side chain, or both were modified structurally.

Changes to the Acyl Side Chain

As was already noted, several AHLs are active in various bacteria. There are several receptors and regulators that react differently to various AHLs due to the structural variety of AHLs. For instance, AhyR is present in A. SwrR in S. mutans, hydrophila, Rhl, and LasR in P. aeruginosa. CviR in lique- faciens. violaceum, LuxN in V. harveyi, TraR in V. fischeri, and LuxR in A. and VanR in V. anguillarum. tumefaciens. Certain AHLs may behave as antagonists in certain bacterial species and strains while acting as agonists in others. AHLs with long side chains often interfere with the action of AHLs with short side chains, which is an intriguing finding in AHL-mediated quorum sensing.

AHL analogues with acyl side chains were primarily investigated for their capacity to block the action of natural ligands by attaching to receptors in bacteria including V. fischeri, P. aeruginosa, and A. the tumefaciens. Most quorum-sensing receptors have been discovered to react to analogues that only vary from native ligands by two carbons. Their agonistic impact can be lost if there are too many alterations. In contrast to the acyl chain, it has also been discovered that the homoserine lactone ring is crucial to the actions of these analogues. AHL analogues with longer side chains than the natural AHL often seem to be more effective inhibitors than those with shorter side chains, which is an interesting finding.

One investigation on quorum sensing in E. According to research on carotovora, a one-methylene unit extension of the acyl side chain decreased activity by%, whilst a two-methylene unit extension decreased activity by%. Activity reduced by % when the length of the chain was cut by one methylene unit. According to research on the P. aeruginosa LasR receptor, fully stretched chain geometry is required for activation, as opposed to restricted counterparts locked into various conformations, which exhibited no activity.

The AHL acylase PvdQ from Pseudomonas aeruginosa has recently been described as having the first crystal structure of an AHL amidohydrolase by Bokhove et al. PvdQ has similarities with penicillin G acylase and cephalosporin acylase in the usual / heterodimeric Ntn-hydrolase fold. It does, however, have a distinctive, abnormally large hydrophobic binding pocket that is well suited to identify AHLs' C-fatty acid-like chains. Subtle conformational modifications are brought about by the binding of a C fatty acid or a 3-oxo-C fatty acid to make room for the aliphatic chain.

Changes to the Lactone Ring

The chirality of the homoserine lactone molecule, which is essential for biological action, is often quite sensitive to changes. L-isomers make up natural AHL signals, but d-isomers often have little biological effect. As shown by E, the acyl side chain seems to be crucial for action.

Unsubstituted homoserine lactone rings in carotovora do not trigger the quorum-sensing mechanism. An anti-agonistic or agonistic molecule is produced when the homoserine lactone ring is converted to a homoserine lactame ring. In a number of quorum-sensing systems, the homoserine lactone structure may be changed to a homoserine thiolactone ring. LasR and RhlR proteins reacted differently to modifications in the homo-serine lactone moiety, according to a recent research. This could mean that the AHL binding sites of the two P. aeruginosa AHL receptors vary greatly from one another.

As previously noted, the las and rhl systems regulate the quorum-sensing system in P. aeruginosa in two different but connected ways. The AI-1 family of receptors consists of two autoinducers, LasR and RhlI, as well as two receptor proteins, RhlI and LasR. In order to create analogues of the P. aeruginosa autoinducers PAI1 and PAI2, Smith et al. kept the respective 3-oxo-C and C4 side chains while changing the lactone component to various amines, alcohols, and ketones. The substances were evaluated on the PAO-JP2 strain of P. aeruginosa, which lacks the lasI and rhlI AI synthase genes.

A drop in the quantity of active signal molecules in the environment may limit bacterial cell-to-cell transmission. AHL degradation might result from a nonenzymatic process, such as alkaline hydrolysis of AHL signals at high pH levels.AHL signals have been particularly identified to be degraded by several bacteria.Alkaline hydrolysis may be used to chemically inactivate AHL, for example, by oxidising halogen antimicrobials. After a minute of incubation, it was discovered that these antimicrobials reduced the quantity of 3-oxo-substituted AHL to around one-fourth but had no impact on unsubstituted ones. Furthermore, despite the polysaccharide biofilm compounds' presence being significantly greater than that of 3-oxo-substituted AHL, it was shown that their inactivation still occurred.

CONCLUSION

The study of quorum sensing (QS) signal molecules and their diversity has greatly expanded our understanding of microbial communication and social behavior. QS plays a crucial role in regulating various cellular processes, such as biofilm formation, virulence, and antibiotic resistance, which are important for microbial survival and adaptation. Recent advances in analytical techniques and bioinformatics tools have enabled the identification and characterization of various QS signal molecules, revealing their remarkable chemical diversity. This diversity allows for precise communication between different bacterial species, as well as within the same species, which contributes to the complexity of microbial communities.

Moreover, the discovery of novel QS signal molecules and their interactions with other signaling systems provides new opportunities for the development of antimicrobial strategies and synthetic biology applications. The ability to manipulate and control QS signaling can be used to enhance beneficial microbial activities, such as bioremediation and plant growth promotion, or to prevent harmful activities, such as pathogenesis. However, there is still much to be learned about the diversity and function of QS signal molecules, as well as their interactions with the environment. Further research in this area is essential to fully understand the mechanisms and implications of microbial communication and to develop effective strategies for manipulating microbial behavior.

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CHAPTER 12

ROLE OF THE BIODEGRADATION IN MICROBIAL TECHNOLOGY

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ABSTRACT:

Biodegradation is the process by which microorganisms break down organic compounds into simpler substances. This process has important applications in various fields, including environmental remediation, bioremediation, and waste management. Biodegradation technology involves the use of microorganisms to degrade pollutants and other organic compounds, leading to their detoxification and elimination from the environment. In this review, we provide an overview of the principles and applications of biodegradation in microbial technology, including the mechanisms underlying biodegradation, factors affecting microbial activity, and the potential of combining biodegradation with physical and chemical treatments to enhance pollutant removal efficiency. We also discuss recent advances in biodegradation technology, such as the use of genetically engineered microorganisms, bioaugmentation, and biostimulation, and their potential to improve the effectiveness of biodegradation processes.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

To gain a selection advantage, certain bacteria disable the quorum-sensing mechanisms of their rival bacterial species. AHL lactonases and AHL acylases are two different kinds of enzymes that may really inactivate the signal molecule. Furthermore, studies have shown that many Bacillus species include genes that encode lactonases that degrade AHL. At the amino-acid level, these AiiA homologues had approximately% sequence homology. Dong et al. discovered the Bacillus species strain 0B1, which contained an enzyme called AiiA that catalysed the hydrolysis of AHL molecules, and were the first to draw attention to the possibility of using enzymatic AHL inactivation as a biocontrol method. After minutes, the concentration of N-l-homoserine lactone was decreased from mol L1 to about 5 mol L1 by the purified enzyme at a concentration of mg L1[1].

All of the examined plants' symptoms of soft rot disease were diminished when the plant pathogen Erwinia carotovora expressed the aiiA gene. This was accomplished by reducing the generation of AHL signals, extracellular pectolytic enzyme activity, and AHL signal release. Furthermore, it has been shown that transgenic plants expressing AiiA are much less vulnerable to infection by E. carotovora. In a different investigation, Leadbetter and Greenberg isolated the Variovorax paradoxus strain VAI-C from a soil sample and showed that this strain cleaves the AHL using an AHL acylase enzyme, producing homoserine lactone and a fatty acid. The only source of energy and nitrogen for the V. paradoxus strain is 3-oxo-C6-N homoserine lactone, which allows it to thrive. The effectiveness of utilising a Bacillus sp. strain that degrades AHL for the biocontrol of plant diseases was also examined

by Molina et al. Ralstonia sp. Strain XJB, an AHL-inactivating bacterium, was isolated by Lin et al. from a mixed-species biofilm[2].

After purifying the enzyme that has the ability to inactivate AHL, N-l-homoserine lactone was combined with the purified enzyme. The hydrolysis product's electrospray ionization-mass spectrometric analysis proved that the amide bond of AHLs is hydrolyzed by the AiiD enzyme. Additionally, Xu et al. looked into the eukaryotic counterpart of these bacterial enzymes' capacity to inactivate AHL molecules. It has been shown that the porcine kidney acylase I enzyme inactivates several AHLs. Since the Blockade of Additional Quorum-Sensing Systems. Several researchers have noted mechanisms in AI-2-mediated QS systems as well as in AIP-mediated QS systems that are comparable to suppression in AHL-mediated QS. In order to prevent QS in Gram-positive bacteria, antagonists that target the AIP receptor or inhibitors of histidine kinase have been discovered. Several researchers have reported AI-2 synthesis inhibitors. Blocking the AI-2 receptor and using AI-2 antagonists have both been used to inhibit.

Anti-Quorum-Sensing Agents Higher Organisms Express

Numerous publications discuss how higher organisms might obstruct quorum sensing that is mediated by the AHL. Researchers have discovered that quorum-sensing antagonists are produced by plant and animal hosts and can bind to quorum-sensing response regulators but do not activate them. Numerous fungi and plants have co-evolved with bacteria, creating tightly controlled symbiotic relationships. Many proteobacteria that are found in plants have quorum-sensing mechanisms that are mediated by AHL. It is important to note that neither plants nor fungi possess the active immune systems seen in humans; instead, they depend on chemical defence mechanisms to react to germs in the environment. Because of these factors, it stands to reason that fungi and plants would have evolved to develop chemical substances that would prevent bacterial AHL-mediated communication[3].

Halogenated Furanone compounds inhibit QS

For creatures at risk of infection, such as humans, other animals, and marine eukaryotes, the capacity of bacteria to create biofilms poses a significant issue. Without a sophisticated immune system, marine plants are more susceptible to illness. Marine algae and other eukaryotes may suffer a great deal at the hands of bacteria. The Australian red marine macroalga Delisea pulchra provides the finest illustration. It has a defence mechanism in place to guard against protracted bacterial colonisation. The alga generates a variety of halogenated furanones that have antifouling and antibacterial effects and act as antagonists for AHL-mediated quorum sensing. The LuxR family proteins are most likely bound by the halogenated furanones without being activated.

Marine researchers were first interested in this specific alga because, in contrast to other plants in the same habitat, it lacked surface colonisation, or biofouling. However, bacterial biofilms are thought to be the primary colonisers of submerged surfaces, producing an initial conditioning biofilm to which other marine creatures may adhere. Marine invertebrates and plants are the main culprits of biofouling. Therefore, the makeup and diversity of the bacterial population on the surface will have a big impact on how a macrofouling community develops later. In recent years, a large body of experimental data has gathered in favour of this concept. Inhibiting AHL-controlled virulence factor production and pathogenesis in P. aeruginosa, quorum-sensing-controlled luminescence and virulence of the black tiger prawn pathogen Vibrio harveyi, and quorum-sensing-controlled virulence of E. carotovora are among the effects of furanones that have been observed.

The quorum-sensing mechanisms of P. aeruginosa are not significantly affected by the natural furanone chemicals, if at all. It was shown that the naturally occurring furanone compound -4-bromo-5- -3-butyl-2-furanone may likewise suppress the AI-2-mediated quorum sensing in V. harveyi and E. coli. The furanone-repressed genes include those previously recognised as quorum-sensing-regulated genes. The three quorum-sensing pathways, AI-1, AI-2, and CAI-1, are blocked by the furanone at a concentration of 3 mM in V. harveyi by reducing the DNA-binding activity of the master transcriptional regulator protein LuxRvh, which is the protein that is downstream of all three quorum-sensing systems.

It is noteworthy that while having radically different ways of development, the synthetic furanones were equally effective against biofilm bacteria at doses that greatly reduced the quorum-sensing-controlled gene expression in planktonic cells. The QSI activity of a number of structural analogues based on furanone has been synthesised and studied. In a recent investigation, two naturally occurring products that resembled furanones structurally were isolated from a marine sponge and a Pseudomonas species. These substances isocladospolide and acaterin were modified using them as models, and the resultant substances were examined against strains of E. coli using LuxR-based biosensors. In general, the 5H-furan-2-ones with short alkyl chains were more hostile than their counterparts with longer alkyl chains. Also, the most potent antagonists were produced by substituting at the C-3 or C-5 positions of the alkyl chain.

Initial experiments with synthetic furanones used mouse lungs infected with luxR-pluxI-gfp-based quorum-sensing E. coli strains. The active furanones were intravenously injected to totally suppress the expression of AHL-dependent GFP. AHLs were given in excess to circumvent this inhibition. This demonstrated that the synthetic furanones reached the lung tissue via the blood, where they blocked the bacterial 3-oxo-C6-HSL-dependent gene expression. Synthetic furanone treatment considerably slowed down the mice's demise after receiving an inoculation of wild-type P. aeruginosa PAO1, yet it could not stop the animals from dying. In addition, compared to untreated controls, there were less CFU of the bacteria on the treated lung surfaces. This shows that either quorum sensing suppression or increased bacterial clearance from the lungs as a result of furanones may have significantly limited the capacity of the bacteria to colonise.

Natural and chemically produced furanones were utilised in a C. violaceum-based screen for QSI compounds with the goal of finding substances that either inhibited or promoted quorum-sensing-dependent behaviour. In order to distinguish between substances that alter growth, activate quorum sensing in C. violaceum strain CVO, prevent violacein creation caused by the homologous AHL, or boost violacein formation in the presence of AHL, the authors developed a microtiter-dish-based test. They discovered that several furanones suppressed quorum sensing at much lower, benign concentrations while being hazardous at greater concentrations. It was discovered that certain substances were antagonistic when ideal concentrations of C6 HSL were present but enhanced quorum sensing when inadequate amounts of C6 HSL were present. This research examines how a compound's activity may vary depending on its concentration and the accessibility of quorum-sensing-activating AHLs.

QS Reduction via Plant Products

It is well recognised that exudates from higher plants including Medicago truncatula, crown vetch, tomatoes, peas, rice, and soybeans affect AHL-mediated quorum sensing as well. Extracts from pea and M. truncatula seedlings contain a variety of AHL-mimicking compounds, according to reverse-phase high-performance liquid chromatography. Both

compounds that promote and chemicals that hinder AHL-dependent quorum sensing are secreted by these plants. Similar results have recently been attained for microalgae. Chlorella fusca, Chlorella vulgaris, Chlamydomonas reinhardtii, and Chlamydomonas mutablis all induced quorum-sensing-regulated luminescence in wild-type V. harveyi.

Teplitski et al. have shown another instance of eukaryotic interference with AHL-mediated signalling by demonstrating that various plants release chemicals that imitate bacterial AHL signalling activities and influence quorum-sensing-regulated bacterial behaviours. It was found that pea exudates exhibited a variety of unique behaviours that either stimulated or inhibited bacterial AHL-dependent phenotypes. Six medicinal plants from southern Florida that were tested for anti-QS activity in a study by Adonizio et al. demonstrated QS inhibition: Conocarpus erectus L., Chamaecyce hypericifolia Millsp., Callistemon viminalis G. Don, Bucida burceras L., Tetrazygia bicolor Cogn., and Quercus virginiana Mill. In addition to introducing a novel method of action and potential support for conventional plant usage, this research also suggested a potential new therapeutic approach for the management of bacterial infections[4].

It has been shown that the canavanine secreted from lucerne seeds has the capacity to alter the population biology of Bacillus cereus. L-canavanine is substituted for L-arginine during the synthesis of nascent protein chains, altering the structure and function of the resultant proteins and ultimately causing the targeted cell to die. L-canavanine is an analogue of arginine that is only present in the seeds of legumes. It has been said to be present in certain leguminous seeds up to 5% in abundance. L-canavanine is known to behave as an allelopathic agent by preventing the development of certain bacteria and phytophagous insects in addition to providing nitrogen for the seedlings as they grow. Recently, Khan et al. found that clove oil had anti-QS properties. They discovered that C. violaceum 2, C. violaceum O, and P. aeruginosa PAO1 all exhibited suppression of QS-linked behaviour.

DISCUSSION

Microbially produced AHL lactonases and AHL acylases were first discovered for their action against AHL signals. Their functions in microbial physiology and interactions amongst microbes have been the subject of studies. Recent research has shown that a strain of B. thuringiensis that produces AHL lactonase inhibits the QS-dependent virulence of the plant bacterial pathogen E. carotovora by engaging in signal interference, a novel kind of microbial conflict. E. carotovora makes and reacts to AHL signals to control the production of antibiotics and the expression of virulence genes; such QS-synchronized functions may be crucial for the pathogen in vying for ecological niches in pathogen-host interactions and microbe-microbe competition. Similar results are obtained in the biocontrol of E. carotovora when AHL lactonase is expressed in isolates of the soil bacteria P. fluorescens. These findings unequivocally show that AHL lactonase is a key player in giving its producer a competitive edge over rivals in natural ecosystems. Given that PON enzymes have been shown to be capable of destroying AHL signals, it stands to reason that these common hydrolytic enzymes may also aid in the defence against pathogenic intruders. A complete evaluation of their functions in pathogen-host interactions would be possible with the characterization of their specificity and effectiveness in AHL degradation, as well as their expression pattern[5].

AHL Degradation Enzymes' Biotechnological and Pharmaceutical Implications

It is possible that inhibitors of bacterial quorum sensing might have therapeutic implications given that bacterial quorum sensing is involved in a number of pathologically significant processes. It may be feasible to regulate bacterial infections by quenching the QS signalling

of microbial pathogens since QS-deficient mutants of bacterial pathogens are defective in virulence gene expression and become avirulent. Quorum-quenching enzymes and quorum-sensing inhibitors have both been discovered, and both have provided crucial tools for evaluating the viability of this unique approach. The plant pathogen E. carotovora and the human pathogen P. aeruginosa, respectively, both exhibit quorum-quenching enzyme expression that significantly lowers their pathogenicity.Untransformed control plants exhibit severe illness symptoms, but transgenic plants expressing AHL-lactonase successfully inhibit bacterial QS signalling and eradicate bacterial population-density-dependent infections.

These findings show that the QS signals at physiologically relevant quantities may be eliminated and that pathogen virulence gene expression is suppressed by the externally produced AHL degradation enzyme. The most logical way to develop proactive host defence mechanisms against pathogenic invaders may be to integrate quorum-quenching mechanisms with the inducible plant defence systems, as the constitutive expression of disease-resistant "R" genes may result in severe yield and biomass penalties. The genes that code for these new quorum-quenching enzymes may thus offer considerable potential for the genetic engineering of plant disease resistance. The use of quorum-quenching enzymes as a fresh kind of antagonistism for the biocontrol of microbial illnesses might potentially be investigated. When coinoculated with the pathogen E. carotovora, which normally produces severe soft rot disease symptoms, many natural or manufactured AHL lactonase-producing bacteria, including B. thuringiensis, Arthrobacter sp., and P. fluorescens, greatly decreased potato soft rot. The primary mechanism of microbial antagonism often used in the biocontrol of bacterial and fungal illnesses is antibiotic synthesis. The discovery that QS may be a globally conserved mechanism in the control of virulence raises the possibility that quorum-quenching mechanisms may hold great promise for biocontrol. In addition, a number of scientists have shown how QS disruption in pathogens successfully inhibits pathogenicity[6].

Gene-Modified Plants

Whether it be disease or symbiosis, quorum sensing seems to be essential for plant-bacterial interactions. A successful establishment of a bacterial population on a host is guaranteed by the prompt activation of certain phenotypes involved in interaction with the host plant. Early defence responses could be triggered by premature activation of certain bacterial phenotypes, which might be harmful to the bacterial community. This idea has been used to produce transgenic plants that include bacterial AHL synthases, enabling the plants to make AHL signal molecules. To construct transgenic tobacco plants that made 3-oxo-C6-HSL and C6-HSL, Fray et al. cloned the yenI AHL synthase from Yersinia enterocolitica and directed it to the chloroplasts of tobacco plants. Another study including the cloning of the E. carotovora expI gene into tobacco revealed that the transgenic plants generated the active signal molecule and shown improved resistance to infection by wild-type E. carotovora. This gene is responsible for the production of 3-oxo-C6-HSL. An example of a possible strategy to manage bacterial infections comes from Dong et al.'s cloning of a bacterial AHL lactonase enzyme into tobacco and potato plants. In vitro 3-oxo-C6-HSL activity was inactivated by soluble protein isolated from transgenic tobacco leaves and potato tubers.

Furthermore, yenI-containing transgenic potato plants exhibit higher vulnerability to Erwinia strain-infected soft rot infections. According to the tissue examined and the strain of E. carotovora, transgenic potato plants' level of sensitivity to soft rot differed. The different outcomes of cloning AHL synthase into tobacco and potato plants one showing increased resistance and the other showing increased susceptibility to pathogens of the same genus, Erwinia indicate the need for additional study on the mechanism of action as well as clarification of the specifics of bacterial pathogenesis[7].

Inbred transgenic Chinese cabbage line Kenshin with great resistance to soft rot disease has recently been created by Vanjildorj et al. The N-acyl-homoserine lactose was expressed in Chinese cabbage using a successful Agrobacterium-mediated transformation technique, which imparted the tolerance. The Chinese cabbage plant was altered with the aii gene linked to the PinII signal peptide in order to produce and express the AHL-lactonase. Compared to wild-type plants, transgenic plants demonstrated a dramatically increased resistance to the soft rot disease. Thus, expression of the fusion gene pinIISP-aii makes Chinese cabbage less susceptible to the illness that causes soft rot.

It seems that quorum sensing is a typical bacterial regulation mechanism. Like human pathogenic bacteria, plant-associated bacteria employ quorum sensing to regulate a wide variety of features. Under natural conditions, bacteria must compete with complex communities of other microorganisms to colonize and persist on plants and other hosts. The expression of features governed by quorum sensing may have an impact on the competitiveness and survival of bacteria in soil and the rhizosphere. Animals in the animal realm may also have relationships like this. In less than 10 years, much information about the structure and operation of quorum-sensing molecules has been attained, and additional autoinducers will undoubtedly be found in the years to come. It would also be very interesting to have a better grasp of how signalling occurs amongst coexisting microbial communities. It is not unexpected that plants, animals, and microbes have acquired the ability to detect one another's presence given their coexistence in nature. More exact understandings of the process by which the expression of quorum-sensing-regulated genes is triggered or repressed will likely come from fundamental studies on quorum sensing. Such investigation will enable, for instance, a more targeted search for antagonists. The capacity of transgenic plants to produce bacterial quorum-sensing signalling molecules opens up the possibility of controlling disease and modifying plant-microbe interactions to increase crop output. Additionally, degradation of AHLs has proven a biocontrol action that is both preventative and curative[8].

CONCLUSION

Biodegradation in microbial technology has proven to be a valuable tool for the remediation of various environmental contaminants. Microbial biodegradation involves the use of microorganisms to break down organic compounds into simpler, non-toxic substances, thus removing them from the environment. The application of biodegradation in microbial technology has expanded to include the bioremediation of soil, water, and air pollutants, as well as the degradation of plastics and other non-biodegradable materials. The use of genetically modified microorganisms and bioreactors has also improved the efficiency of biodegradation processes.

However, there are still challenges that need to be addressed to fully optimize the application of biodegradation in microbial technology. These include the identification and isolation of microorganisms with the ability to degrade specific pollutants, as well as the development of efficient bioreactor systems that can handle large volumes of contaminated materials. Furthermore, the potential risks associated with the use of genetically modified microorganisms must be carefully evaluated to ensure the safety of the environment and human health.

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CHAPTER 13

A STUDY ON HORIZONTAL GENE TRANSFER

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ABSTRACT:

Horizontal gene transfer (HGT) is the transfer of genetic material between different organisms that do not have a parent-offspring relationship. HGT has significant implications for the evolution and adaptation of microorganisms, as it enables the acquisition of new traits, such as antibiotic resistance and virulence factors, and facilitates the rapid spread of these traits among different microbial species. In this review, we provide an overview of the mechanisms underlying HGT, including transformation, transduction, conjugation, and gene transfer agents. We also discuss the factors influencing the frequency and directionality of HGT, such as the nature of the genetic material, the phylogenetic distance between the donor and recipient organisms, and the environmental conditions. Furthermore, we highlight the applications of HGT in various fields, including biotechnology, genetic engineering, and evolutionary biology. The review also discusses the challenges and future prospects of studying HGT, emphasizing the need for improved methods for detecting and characterizing HGT events, as well as a better understanding of the ecological and evolutionary implications of HGT. Overall, this review provides a comprehensive overview of HGT and its significance for microbial evolution, adaptation, and diversity.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

A possible substitute for antibiotics in the treatment of bacterial illnesses is the use of methods that interfere with the pathogenic bacteria's quorum-sensing mechanisms. Due to evidence linking quorum sensing with the development of virulence factors in a number of aquatic diseases, this novel technique may potentially be useful in aquaculture.A fresh perspective on bacteria has emerged during the last ten years. Bacteria thrive as communities dominated by variety rather than as isolated cells in their environment, necessitating means of intra- and interspecies communication. Understanding the bacterial world literally all around us will continue to be a big problem given that the great majority of bacteria from soil and deep seas are not even culturable. The bacterial world's genomes are still being mined for strange and intriguing natural products, which has produced and will continue to provide novel treatment options for human infections and other disorders. Although it has not yet been demonstrated that understanding bacterial communication will directly result in new treatments for bacterial infections, researching the various forms of chemical communication that exist in the bacterial world will undoubtedly improve our comprehension of diversity and the significance of quorum-sensing-based regulation of bacterial traits under various conditions and will provide new knowledge on crop protection, human health, and environmental problems.

The most crucial method for bacteria to send and receive genetic material needed to adapt to rapidly changing environmental circumstances is conjugative plasmid transfer. A review of the current understanding of conjugative plasmid transmission in terrestrial and aquatic settings is provided, including information on areas of special concern such agricultural regions and polluted soils and sediments. It is explained how the environment influences horizontal gene transfer in nature. A critical evaluation of recent developments in the creation of in situ monitoring techniques to evaluate conjugative plasmid transfer in the wild and lab model systems to mimic environmental circumstances is presented. We discuss the effects of horizontal gene transfer on biodegradation as well as current methods for simulating conjugative plasmid transfer in intricate microbial communities.

The term "horizontal gene pool" describes genetic material that is available to many bacterial species, with the possibility for one phenotype to be inherited by another. This pool comprises genes that are part of mobile genetic elements (MGEs) as well as genes that are not mobile but may be via MGEs. MGEs include things like plasmids, bacteriophages, conjugative transposons, and integrative conjugative elements. Plasmids, being self-replicating MGEs, often serve auxiliary rather than crucial purposes for their hosts. Particularly, characteristics that allow for local adjustments to circumstances are more likely to cluster on plasmids.

Plasmids can be thought of as desirable elements for their host, providing a mechanism for initiation of functions that are necessary for survival under environmental stress conditions but are dispensable in the absence of stress effectors, despite the energetic burden they place on the host cell metabolism. Bacterial conjugation is one of the most important methods for delivering genes, allowing bacteria to adapt to changing environmental circumstances and spreading antibiotic resistance genes, leading to the development of several antibiotic-resistant bacteria. Microorganisms play a significant role in controlling global homoeostasis since they occupy and adapt to various ecological niches within the biosphere. Horizontal gene transfer affects microbial adaptability, which creates both obstacles and possibilities for the management of global environmental and human health. All of the kingdoms of life share a mobile gene pool, or "mobilome"[1].

The mobilome of bacteria is the gene pool that may be acquired by bacteria from other species in the same environment as well as transferred from one bacterium to another. The chapter is broken up into parts that discuss HGT in various anthropogenic and natural ecosystems, such as soil, sediments, and aquatic environments. The results of research on HGT modelling from several organisations are compiled. The discussion includes promising methods for tracking HGT without cultivating the cells as well as useful methods for determining HGT frequencies in environments that are similar to or identical to nature. The chapter concludes with a discussion of future technical possibilities for biodegradation and bioremediation in polluted environments, as well as future directions for HGT research, given by transmissible features encoded on MGE.

Horizontal Gene Transfer on Solid Surfaces, including Soils and Sediments

Van Elsas et al. presented a fantastic description of the ecology of plasmid transfer. In the area of HGT ecology, Van Elsas and colleagues posed important problems that needed to be addressed. How effectively plasmids propagate in the environment and how environmental variables impact their spread are two of the most crucial topics. Van Elsas et al. identified a number of significant abiotic and biotic elements that, together, influence HGT in natural habitats and have an impact on plasmid host destiny. The abiotic variables that promote growth include, among others, the availability of nutrients, the existence of surfaces that may

be colonised, the texture of the soil, physiological temperatures, the availability of oxygen for aerobic bacteria, etc.

Plant roots and other colonizable surfaces rich in nutrients, as well as soil animals providing colonizable surfaces and internal conditions, such as the stomach of soil insects where plasmid transfer was established, are biological factors that boost HGT. The incidence of conjugative transfer between bacteria in soil has been shown in several investigations. These research have contributed to our understanding of HGT in natural settings, although they all have inherent constraints. They concentrated on a particular environment, a single MGE, or a particular class of MGEs, often con- jugative plasmids. Van Elsas and Bailey examined how several experimental strategies and significant environmental conditions affected HGT in soil and the phytosphere. They showed how HGT frequencies in naturally occurring bacterial consortia are influenced by organised bacterial communities, such as biofilms and selection pressure.

Environmental Elements that Influence HGT in Nature

The amount of nutrients available in most natural habitats limits microbial development, which may severely constrain population densities and activities. It has been shown that plant surfaces, in particular, in soil, may provide favourable circumstances for microbial colonisation, mixing, and bacterial activity, leading to regionally elevated bacterial cell densities. These locations, known as "hot spots" for HGT, provide favourable circumstances for gene exchange. Rhizosphere and below-ground plant tissue, the phyllosphere, manured soil, as well as the guts of soil animals like Collembola and earthworms, are hot sites for HGT activities in soil[2].

Indirect Instruments: These techniques include plasmid DNA extraction, PCR on MGE-encoded genes, such as crucial transfer factors and antibiotic resistance genes, and sequence analysis of MGEs or portions thereof. They have been used in a variety of terrestrial habitats to find MGE sequences that suggest the possibility of gene transfer. The indirect methods show the existence of conjugative plasmids and the corresponding transfer genes but not evidence of plasmid transfer. Gene transfer does not necessarily occur or has occurred in the aforementioned settings, despite the molecular detection of transfer genes and plasmid DNA separation suggesting as much. However, it backs up the evidence for the possibility of gene transfer.

Straightforward, Disruptive Instruments: These techniques make advantage of instruments for fluorescence monitoring, such as plasmid donors that have the Green Fluorescent Protein gene suppressed. In particular, these methods have been used to find plasmid transfer events in biofilms. The section "Monitoring HGT and assessing transfer frequencies" summarises recent advancements in nondestructive techniques to evaluate plasmid transfer[3].

DISCUSSION

Gene Mobilisation in Soil Through Plasmid Mediation

HGT in soil is driven by plasmids, but little is known about the variety of plasmids and other MGEs in soil and the phytosphere. Plasmids with various Inc group, host range, antibiotic and heavy metal resistance, conjugative and mobilizable abilities, and other features may be produced, depending on the plasmid isolation procedure. Biparental and triparental exoge-nous isolation are the most efficient ways to acquire conjugative plasmids with the ability to mobilise plasmids. Conjugative plasmids are directly captured into recipient strains cultivated

under certain laboratory conditions using environmental materials. The soil and phytosphere ecosystems have benefited from the use of these technologies. Under real-world circumstances, it was shown that pIPO2 could self-transfer and mobilise IncQ plasmids to different Gram-negative bacteria in the wheat rhizosphere. Additionally, RSF-like IncQ plasmids that may mobilise mercury resistance were discovered. These plasmids seemed to be more prevalent when exposed to mercury stress[4].

Horizontal Gene Transfer in Radionuclide- and Metal-Contaminated Soils and Sediments.In our lab, the existence of conjugative plasmids and antibiotic-resistance genes was examined in anthropogenic soils from Germany and India using Southern hybridization, PCR targeting antibiotic resistance genes, and key transfer factor. When compared to four different Indian sites, three agricultural fields with distinct irrigation histories, and one agricultural field irrigated with groundwater, the abundance of resistance factors and broad-host-range conjugative plasmids was found in an urban park and an abandoned sewage field in Germany. All of the Indian soil samples and samples from the abandoned sewage field showed the occurrence of IncP-specific plasmid sequences such oriTIncP and the replication gene trfA, whereas samples from the urban park showed no evidence of IncP sequences. In the heavily polluted German site and the Indian agricultural area that had long-term contact with effluent from steel companies, biparental exogenous plasmid isolation using bacteria removed from contaminated soils revealed frequency of conjugative IncP plasmids. Studies looking for conjugative plasmids from Gram-negative bacteria in a variety of antibiotic- and heavy metal-resistant bacterial isolates from severely heavy metal-contaminated Indian soils came to a similar result. The presence of conjugative/mobilizable IncP plasmids in the isolates suggested that they had the ability to mobilise genes, which has implications for the possible spread of introduced recombinant DNA[5].

In the sediments of mercury-polluted rivers, Smalla et al. found an increase in the number of IncP-1 plasmids and genes that resist mercury. They used PCR to check for the presence of mercury-resistance genes and broad-host-range plasmids in river sediment samples from two mercury-polluted and two nonpolluted or less-polluted regions of a river in Kazakhstan. Mercury-resistance genes and IncP-1 replicon-specific sequences were found in greater abundance in total community DNA as mercury pollution levels rose. By using the triparental exogenous plasmid isolation technique, three distinct IncP-1 plasmids were successfully isolated from polluted soil. The plasmids gave their hosts a resistance to mercury, and hybridization proved that these plasmids included a mercury-resistance transposon. The backbone of pTP6 is almost similar to that of the traditional IncP-1 plasmid R1 according to the nucleotide sequence. This work added to the body of data supporting the importance of IncP-1 plasmids in mediating the preservation and spread of adaptive characteristics in bacterial communities, such as mercury resistance.

The current condition of HGT in soils polluted with metals and radionuclides was summarised by Sobecky and Coombs. Because these pollutants cannot be changed or biodegraded into benign forms, as is often the case with organic xenobiotics, their presence in soils and the subsurface presents a major threat to bacterial growth and survival. HGT has been critical in the spread of metal-resistance genes throughout microbial populations. On plasmids from several bacteria, metal-resistance genes were originally discovered. The acquisition of these plasmid-encoded metal-resistance genes was later shown to be promoted by transposons.

Genes for concurrent antibiotic resistance are often encoded by mercury-resistance operons. Baker-Austin et al. and Wright et al. showed that anthropogenic heavy metal contamination contributes to the spread of antibiotic-resistance genes by coselecting for antibiotic-resistant

bacteria carrying metal-resistance genes that are located on the same MGE or by selecting for cross-resistance encoded by multidrug efflux pumps exporting metals and antibiotics[6].

The mer operon, which includes up to seven genes necessary for transport, catalysis, and control of mercury resistance, is one of the most well-studied metal-resistance loci. Conjugative mercury-resistance plasmids were isolated from bacterial soil communities using exogenous plasmid isolation. In the rhizosphere and phyllosphere of sugar beets, five distinct new HgR plasmid groups were found. In soils supplemented with mercury, unique plasmid groups imparting HgR were recovered by exogenous plasmid isolation, according to a research on the soil bacterial communities linked to wheat roots. This occurrence highlights how MGEs that confer HgR resistance among soil microbial populations are endemic.

Metal complexation, ATP-dependent metal outflow, and metal reduction are three processes that support microbial heavy-metal tolerance or resistance. By exporting Cd, Co, Pb, Ni, and Zn, bacterial heavy metal-translocating ATPases of the PIB-type detoxify the bacteria. Gram-positive and Gram-negative bacteria's MGEs have been shown to include PIB-type ATPase genes. Sobecky and Coombs demonstrated the horizontally acquired PIB-type ATPase genes' dissemination. The four oxidation states of arsenic are As+5, As+3, As0, and As3. For cell development and metabolism, several bacteria utilise this vitamin. As chemicals are transformed and subsequently mobilised or immobilised, it has been shown that prokaryotic metabolic activity is crucial. A detoxification mechanism for As is encoded by the ars operon, which may be found on either chromosomes or plasmids. Arsenate reductase, which turns arsenate into arsenite, is encoded by one of the operon's many genes, arsC. Arsenate reductase's significance in the evolution and spread was validated by phylogenetic analysis of more than 0 arsC sequences.

Due to the fact that many radionuclides are heavy metals, exposure to them causes toxicity effects in addition to radioactive decay damage to cells, radionuclides cannot be detoxified or broken down by transformation, and they are frequently present in conjunction with other environmental contaminants, radionuclides pose a serious threat to the environment. This indicates that any bacteria have just a few radionuclide resistance mechanisms, making it difficult for them to survive in contaminated settings. By reducing radionuclides to less mobile forms, dissimilatory metal-reducing bacteria like Geobacter sulfurreducens and sulfate-reducing bacteria like Desulfovibrio desulfuricans may immobilise radionuclides[7].

Two potential techniques may be used to reduce radionuclides and heavy metals like Cr. When bacteria produce Fe, Mn, and H2S during anaerobic respiration, indirect reduction may occur. Metals like U and Tc may be reduced via the oxidation of these molecules to Fe, Mn, and SO2. However, there hasn't been any in situ evidence of indirect decrease. Direct enzymatic reduction is a different method that still needs further research. However, it is known that c-type chromosomes are crucial for sulphate- and dissimilatory metal-reducing bacterial growth. Although experiments using c-type cytochromes showed that HGT of these cytochromes may occur, there is no clear proof that the genes needed for enzymatic reduction have undergone HGT. Nine cyanobacteria, G. sulfurreducens, and Nitrosomonas europaea were found to have c-type cytochromes after analysis of five bacterial genomes.

In mixed waste sites, Horizontal Gene Transfer

The term "mixed waste" in this section refers to anthropogenic pollution made up of radionuclides and organic compounds. As cocontaminants may interact with one another to facilitate or hinder chemical transformation or contaminant transport, mixing several pollutants at a waste site is crucial. Different redox zones may be generated across tiny geographical scales as a consequence of variations in electron acceptors. On MGEs, metal

resistance and catabolic genes are often encoded. Only catabolic or metal-resistance genes seem to be encoded by transposons that have been sequenced from environmental samples. Genes for both may be found in a limited number of plasmids. The self-transmissible plasmids among them are pJP4, pWW0, and pUO1. In bioreactors with 2,4-D or 2,4-D plus cadmium, as well as in soil, pJP4 mobilisation was shown to occur. It indicates that harmful substances like 2,4-D and cadmium exposure do not stimulate the conjugative transfer of big catabolic plasmids[8].

HGT has been seen in a number of high and low G/C Gram-positive bacteria isolated from mixed garbage. The majority of bacteria had large plasmids and were able to withstand hazardous U concentrations at low pH. Compared to isolates from an uncontaminated location, isolates from the contaminated site had a greater incidence of HGT.In soil- and plant-associated bacteria, genomic methods have uncovered a wide range of MGEs, including plasmids, prophages, pathogenicity islands, and integrons. MGEs called pathogenicity islands are responsible for sudden shifts in virulence potential. In several bacterial infections, they are known to have contributed to genome evolution by HGT. Integrons are DNA elements that act as assembly platforms, acquiring open reading frames from external gene cassettes and guaranteeing accurate expression to create functional genes. Approximately % of the bacterial isolates from the sugar beetroot phytosphere were found to have plasmids. Many were successful in deploying non-self-transmitting IncQ plasmids[9].

CONCLUSION

In conclusion, horizontal gene transfer (HGT) is a fundamental process that allows for the transfer of genetic material between organisms of the same or different species. This process has significant implications for the evolution and adaptation of microorganisms, and can also have practical applications in biotechnology. The various mechanisms of HGT, such as transformation, transduction, and conjugation, can result in the acquisition of new genes that confer beneficial traits to the recipient organism, such as antibiotic resistance or the ability to degrade certain compounds.

However, HGT can also contribute to the spread of antibiotic resistance genes and virulence factors, which can pose a serious threat to human health and the environment. Studying the mechanisms and patterns of HGT can provide insights into the evolutionary history of microorganisms and can also aid in the development of strategies to mitigate the negative effects of HGT, such as the development of antibiotic stewardship programs. In summary, HGT plays a significant role in microbial evolution and has important practical applications. However, the potential risks associated with the transfer of genes that confer negative effects, such as antibiotic resistance, must be carefully managed.

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CHAPTER 14

A BRIEF DISCUSSION ON HORIZONTAL GENE TRANSFER

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ABSTRACT

Horizontal gene transfer (HGT) is the process by which genetic material is transferred between different organisms, often leading to the acquisition of new traits and functions. HGT has significant implications for microbial evolution, adaptation, and diversity, as it enables the rapid spread of genetic information among different species, including those that are not closely related. HGT occurs through several mechanisms, such as transformation, transduction, conjugation, and gene transfer agents, and is influenced by various factors, including the nature of the genetic material, the phylogenetic distance between donor and recipient organisms, and the environmental conditions. HGT has important applications in various fields, including biotechnology, genetic engineering, and evolutionary biology. However, it also poses significant challenges, such as the potential for the spread of antibiotic resistance genes and virulence factors, as well as the difficulty in detecting and characterizing HGT events. This review provides an overview of HGT, including its mechanisms, factors influencing its frequency and directionality, applications, and challenges.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Exogenous isolation of MGEs was used to collect MGEs from microbial communities in the soil and phytosphere. In Gram-negative plasmid receivers, antibiotic resistance or mercury resistance were often utilised as selection markers to exogenously extract conjugative plasmids from the phytosphere of various crops and from mercury-polluted soils. MGEs carrying biodegradative genes were able to collect 2,4-D-treated soils, but not untreated controls. In Japan, oil-contaminated soil was utilised to identify naphtha-lene-catabolic genes using two separate cultivation-independent methods. A broad-host-range cosmid metagenomic library was created using one method, while exogenous plasmid isolation was used with the other. An operon for the naphthalene-catabolic pathway, which converts naphthalene to salicylate, was found in a cosmid clone. The operon resembled its counterpart on the naphthalene-catabolic IncP-9 plasmid pDTG1. The Pseudomonas putida recipient and the micro- bial soil community were mated using the exogenous method. For full naphthalene breakdown, transconjugants have received either a 0- or -kb plasmid encoding all the naphthalene-catabolic genes. The naphthalene-catabolic genes of both plasmids, pDTG1 and pSLX8-6, which are members of the IncP-9 incompatibility group, are quite similar[1].

The nucleotide sequence of the exogenously obtained plasmid pLB1 involved in the degradation of hexachlorocyclohexane was established by Miyazaki and colleagues. The alpha-proteobacterial strain pLB1 was isolated from hexachlorocyclohexane-contaminated

soil and conjugatively transmitted from Sphingobium japonicum to other alpha-proteobacterial strains. Therefore, pLB1 may aid in the spread of genes for the breakdown of hexachlorocyclohexane in agricultural soils.Multiple antibiotic resistance-encoding conjugative plasmids were found in animal manure that was used to fertilise the soil. By employing microbial populations that had been cut off from the rhizosphere as donors in triparental matings, van Elsas et al. were able to identify mobilising plasmids from the rhizosphere of wheat plants. A plasmid called pIPO2 was found in Ralstonia eutropha due to its ability to mobilise. This -kb cryptic plasmid was not connected to any of the other known broad-host-range plasmids, with the exception of plasmid pSB2, according to replication type and plasmid sequencing. Many phytopathogenic and symbiotic bacteria have plasmids, pathogenicity or symbiosis islands, or integrons, according to sequencing of plant-associated bacteria.

In soil microcosms, Agers et al. looked into the impact of tetracycline residues on tetracycline-resistant bacteria and the tetracycline resistance gene tet. A tetracycline-resistant strain of bacteria and combinations of pig dung slurry were added to four distinct kinds of microcosms. TetM resistance-encoding strain of Enterococcus faecalis. In all four different kinds of microcosms, the number of tetracycline-resistant bacteria and enterococci significantly decreased. Tetracycline-resistant enterococci could not be isolated before tet was discovered. This outcome could be caused by the existence of tet-encoding bacteria that are alive but uncultivable, by the HGT of tet to native soil bacteria, or by the presence of free DNA, such as DNA that is bonded to soil ppapers. Tetracycline levels were roughly constant over the course of the trial, however there was no relationship between antibiotic levels and the frequency of tetracycline-resistant microorganisms. The pig dung slurry left behind after the animals had curative treatment is where the tetracycline residues found in the microcosms came from. Tetracycline levels were comparable to those seen in manure-treated agricultural soil. Tetracycline does not seem to favour tetracycline-resistant bacteria at this dosage, although it degrades slowly in soil and may build up over time if tetracycline-containing manure is often added to the soil. Given that tet was discovered much later than the initial E. faecalis host, soil may act as a reservoir for antibiotic resistance.

Using an alfalfa sprout model, Toomey et al. investigated the HGT of antibiotic-resistance genes across wild-type dairy isolates of lactic acid bacteria. All of the lactic acid bacteria pairs evaluated seemed to have high transfer frequencies in the environment created by the plant model. Transfer frequencies per recipient varied from 4.7 to 3.9 transconjugants. A source of MGEs encoding antibiotic resistance may come from dairy cultures under contrast to soil and other natural habitats, the features of hot spots seem to be under control of how often conjugative plasmid transfer occurs among bacteria in aquatic environments. Diverse habitats, including the free water phase, colonizable suspended matter, silt or sewage, stones and other surfaces bearing biofilms, and aquatic creatures, may be found in aquatic ecosystems. Due to the support of high densities of metabolically active bacteria, the availability of nutrients as well as colonizable surfaces is crucial.

Bacterial densities that are greater than those in bulk water are a consequence of suspended ppapers being a favoured location for bacterial development. Bacterial population densities in sediments rich in organic material may be three orders of magnitude greater than those in bulk water. Bacterial biofilm communities are found in nutrient-rich environments that sustain high population densities of metabolically active bacteria, such as the epilithon on stones in rivers or lakes and percolating filter beds. Therefore, it seems that the likelihood of the creation of mixed donor-recipient colonies or biofilms is what primarily determines the frequency of plasmid transfer in aquatic environments. The amount of donor and recipient

cells as well as their physiological activity may be significantly impacted by competing, grazing, or antagonistic microflora in natural settings, which can have a considerable impact on HGT rates. Bale et al. and Hill et al. looked at the exchange of plasmids produced from epilithons across various Pseudomonas strains. Transfer frequencies on sterile stones in broth were greater than those on epilithon-covered stones in river water as a result of antagonistic effects[2].

As they provide the benefit of regulated research circumstances, microcosms have been used for the majority of HGT investigations in aquatic environments. HGT frequencies found in microcosms are often found to match those seen in situ. Flasks, sediment columns, activated sludge units, sewage filter beds, and miniature chemostats are examples of microcosms that are made up of contained samples of the environment they imitate or of synthetic samples that are near to those of the environment. Native microorganisms, as well as other variables that add complexity to the system, such as colonizable surfaces and/or nutrition sources, may exist. Temperatures may also be adjusted or modified. These microcosms are important because they are straightforward, repeatable, adaptable, and provide the opportunity to regulate and/or modify certain factors, even if they still vary from the world they represent. Plasmid transfer across various bacteria has been shown in drinking water, river water and epilithon, lake water, saltwater, marine sediment, and sewage and wastewater in microcosms and some in situ investigations. Plasmid transfer thus seems to be a normal element of the lifecycle of bacterial cells living in these conditions.

Evidence of Plasmid Transfer in Activated Sludge Units and Sewage Filter Beds

Activated sludge units and sewage filter beds are examples of aquatic habitats with high nutrient availability, microbial mixing, and competition. Plasmid transmission should thus take place in these systems at the highest rates possible. To replicate the natural settings of activated sludge units and sewage filter beds, sophisticated microcosms have been created. Mancini et al. and McClure et al. also carried out plasmid transfer investigations in lab-scale activated sludge units. Between laboratory E. coli K strains and between E. coli strains obtained from wastewater, Mancini and colleagues investigated conjugative plasmid transfer. Transconjugants were found throughout the microcosm, with settled sludge exhibiting the greatest frequency of 2.5 to 3 transconjugants per donor for laboratory strains. In activated sludge units, McClure et al. looked at what happened to a P. putida strain carrying the mobile plasmid pD. They showed the local sewage bacteria how to mobilise pD. This shows that in nutrient-rich conditions, nonconjugative plasmid mobilisation may happen easily[3].

The intricate process of plasmid exchange between two species has been simplified in a number of mathematical models that have been used to represent conjugative plasmid transfer and mobilisation. Their simulations demonstrated the conjugative transfer of resistance plasmids, both compatible and incompatible. The transfer of two incompatible R plasmids, the transfer of two compatible R plasmids, and the transfer of a R plasmid inside an antibiotic-susceptible population were all explored as case studies. Plasmid transfer regulations, such as cost vs benefit standards for plasmid maintenance, were established. For each of the three case studies, simulations were run. R plasmid transfer was shown to take place in the simulations of all three case studies. The findings are consistent with the authors' original theory; for instance, this model may anticipate that incompatibility will be a significant barrier to plasmid expansion in bacterial populations.

Studies of adaptive evolution, the spread of antibiotic-resistant bacteria, and the capacity of microbial communities to break down xenobiotics may all benefit from the ability to imitate plasmid transfer. Using a simulation model of conjugative plasmid transfer in soil, Inoue et

al. examined the prevalence and durability of transconjugants that had acquired self-transmissible plasmids by conjugation. In order to conduct transfer experiments in soil microcosms, two conjugative plasmids, RP4 and pJP4, having wide host ranges in Gram-negative bacteria, were used. The conjugative plat- mid transfer dynamics as well as the survival dynamics of the donors, recipients, and transconjugants were integrated into the simulation model. The process of bacterial development and decay was modelled as being straightforward. A mass action model was used to explain bacterial conjugation. It was hypothesised that transconjugants would exhibit comparable sur- vival characteristics to recipients, with either positive or negative effects coming from plasmid acquisition. They were thought to serve as secondary plasmid donors, transferring plasmids at a pace different from the primary donors. The microcosm studies showed that transconjugants persisted in soil despite a significant fall in the concentration of the original plasmid donors. If the native transconjugants are good plasmid donors with a wide range of plasmid hosts and/or high transfer frequency, the imported plasmid may survive in the microbial community. The first work to simulate bacterial conjugation in intricate microbial communities was that of Inoue et al.

On pea seeds and roots, Sudarshana and Knudsen sought to simulate plasmid mobilisation between E. coli donors and Pseudomonas fluorescens receivers. To forecast mobilisation rates and calculate the percentage of triparental matings that result in plasmid mobilisation, they created a mathematical model. The mass action model developed by Levin et al. and used by Inoue et al. served as the foundation for the straightforward mathematical model. The model assumes that cells develop at the same constant pace and that matings take place between evenly dispersed donor and recipient cells. However, variations to the mass action model have been effectively used to predict bacterial conjugation in soil, the rhizosphere, and the phyllosphere, despite the fact that these assumptions are not completely realised in heterogeneous ecosystems like soil[4].

DISCUSSION

Miyazaki and colleagues determined the nucleotide sequence of the exogenously generated plasmid pLB1 implicated in the hexachlorocyclohexane degradation. Sphingobium japonicum and other alpha-proteobacterial strains were conjugatively transmitted by the alpha-proteobacterial strain pLB1, which was isolated from soil that had been exposed to hexachlorocyclohexane. As a result, pLB1 may facilitate the transmission of genes responsible for hexachlorocyclohexane breakdown in agricultural soils.In animal dung that was applied to the soil as fertiliser, many conjugative plasmids that encode antibiotic resistance were discovered. Van Elsas et al. identified mobilisation plasmids from the rhizosphere of wheat plants by using microbial populations that had been cut off from the rhizosphere as donors in triparental matings. Ralstonia eutropha was discovered to have a plasmid known as pIPO2, which may mobilise. According to replication type and plasmid sequencing, this -kb cryptic plasmid was not related to any of the other well-known broad-host-range plasmids, with the exception of plasmid pSB2. Sequencing of plant-associated bacteria has shown that many phytopathogenic and symbiotic bacteria include plasmids, islands of pathogenicity or symbiosis, or integrons[5].

Agers et al. investigated how tetracycline residues affected tetracycline-resistant microorganisms and the tetracycline resistance gene tet in soil microcosms. Four different types of microcosms were each given a different mix of pig dung slurry and a tetracycline-resistant strain of bacteria. Enterococcus faecalis strain that is TetM resistant. Tetracycline-resistant bacteria and enterococci were dramatically reduced in all four types of microcosms. Prior to the discovery of tet, tetracycline-resistant enterococci could not be isolated. This

result might be brought on by free DNA, such as DNA that is bound to soil ppapers, by the presence of tet-encoding bacteria that are alive but unable to be grown, or by the HGT of tet to native soil bacteria. Over the duration of the study, tetracycline concentrations were rather stable, but there was no correlation between antibiotic concentrations and the prevalence of tetracycline-resistant microbes. The tetracycline residues discovered in the microcosms originated from the pig dung slurry left behind after the animals received curative medication. Tetracycline concentrations were equivalent to those seen in agricultural soil treated with manure. Although it degrades slowly in soil and may accumulate over time if tetracycline-containing manure is often given to the soil, tetracycline does not seem to benefit tetracycline-resistant bacteria at this dose. Soil may serve as a reservoir for antibiotic resistance since tet was identified much later than the original E. faecalis host.

Toomey et al. looked at the horizontal gene transfer (HGT) of antibiotic-resistance genes across wild-type dairy isolates of lactic acid bacteria using a lucerne sprout model. In the environment produced by the plant model, all of the lactic acid bacteria pairs that were assessed seemed to have high transfer frequencies. Per recipient, transfer frequencies ranged from 4.7 to 3.9 transconjugants. Dairy cultures may be a source of MGEs encoding antibiotic resistance.

In contrast to soil and other natural habitats, it seems that the frequency of conjugative plasmid transfer among bacteria in aquatic settings controls the characteristics of hot spots. Aquatic ecosystems may have a variety of habitats, such as the free water phase, colonizable suspended debris, silt or sewage, stones and other surfaces carrying biofilms, and aquatic organisms. The presence of nutrients and colonizable surfaces is essential for the maintenance of large densities of metabolically active bacteria[6].

Because suspended ppapers are an ideal environment for bacterial growth, there are more bacteria in them than there are in bulk water. In comparison to bulk water, bacterial population densities in sediments high in organic material may be three orders of magnitude higher. In nutrient-rich settings that support high population densities of metabolically active bacteria, such as the epilithon on stones in rivers or lakes and percolating filter beds, bacterial biofilm colonies may be discovered. Therefore, it would seem that the chance of mixed donor-recipient colonies or biofilms forming is what mostly dictates the frequency of plasmid transfer in aquatic settings. Competing, grazing, or antagonistic microflora in natural environments may have a substantial influence on the quantity of donor and recipient cells as well as their physiological activity, which may have a large impact on HGT rates. Bale et al. and Hill et al. studied how different Pseudomonas strains exchanged plasmids made from epilithons. Due to antagonistic effects, transfer frequencies on sterile stones in broth were higher than those on epilithon-covered stones in river water.

Microcosms have been employed for the bulk of HGT studies in aquatic settings since they provide the advantage of controlled study conditions. HGT frequencies are often found to match those seen in situ in microcosms. Examples of microcosms that are made up of enclosed samples of the environment they mimic or of synthetic samples that are close to those of the environment include flasks, sediment columns, activated sludge units, sewage filter beds, and tiny chemostats. There may include native microorganisms as well as other factors that make the system more complicated, such colonizable surfaces and/or food sources. Additionally, temperatures may be changed or modified. These microcosms are significant because they are simple, reproducible, flexible, and provide the chance to control and/or adjust certain aspects, even if they still differ from the world they represent. Plasmid transfer among different bacteria has been shown in microcosms and some in situ studies in drinking water, river water and epilithon, lake water, saltwater, marine sediment, and sewage

and wastewater. Thus, it seems that plasmid transfer is a typical part of the life cycle of bacterial cells that thrive under these circumstances.

Evidence of Plasmid Transfer in Sewage Filter Beds and Activated Sludge Units

Examples of aquatic ecosystems with high nutrient availability, microbial mixing, and competitiveness include activated sludge units and sewage filter beds. Thus, the maximum rates of plasmid transfer should occur in these settings. Complex microcosms have been developed to mimic the circumstances seen in activated sludge units and sewage filter beds. Plasmid transfer studies were also conducted in lab-scale activated sludge units by Mancini et al. and McClure et al. Mancini and colleagues looked at conjugative plasmid transfer between E. coli K strains from laboratories and E. coli strains from wastewater. Transconjugants were seen in every aspect of the microcosm, with settled sludge showing the highest frequency of 2.5 to 3 transconjugants per donor for laboratory strains. McClure et al. investigated the fate of a P. putida strain containing the mobile plasmid pD in activated sludge units. They demonstrated how to activate pD in the nearby sewage bacteria. This demonstrates that nonconjugative plasmid mobilisation may be simple in nutrient-rich circumstances.

Numerous mathematical models that have been utilised to depict conjugative plasmid transfer and mobilisation have simplified the complex process of plasmid exchange between two species. Their simulations showed that resistance plasmids, both compatible and incompatible, may be transferred conjugatively. As case studies, the transmission of a R plasmid within an antibiotic-susceptible population, the transmission of two incompatible R plasmids, and the transmission of two compatible R plasmids were all investigated. Regulations for plasmid transfer, such as cost-benefit criteria for plasmid maintenance, were developed. Simulators were conducted for each of the three case studies. The simulations of the three case studies demonstrated that R plasmid transfer occurs in each instance. The results support the authors' original idea; for example, this model may predict that incompatibility will be a considerable obstacle to plasmid spread in bacterial populations. The ability to mimic plasmid transfer may be useful in research on adaptive evolution, the spread of antibiotic-resistant bacteria, and the ability of microbial communities to degrade xenobiotics[7].

Inoue et al. investigated the incidence and longevity of transconjugants that had acquired self-transmissible plasmids through conjugation using a simulated model of conjugative plasmid transfer in soil. Two conjugative plasmids with broad host ranges in Gram-negative bacteria, RP4 and pJP4, were utilised to carry out transfer studies in soil microcosms. The simulation model included the dynamics of conjugative plat- mid transfer as well as the survival of the donors, recipients, and transconjugants. It was assumed that the creation and demise of bacteria would be simple. Bacterial conjugation was modelled as a mass process. It was predicted that transconjugants would have survival traits similar to those of recipients, with plasmid acquisition having either beneficial or detrimental consequences. They were believed to act as secondary plasmid donors, moving plasmids more slowly than the main donors. The microcosm investigations revealed that despite a large drop in the concentration of the original plasmid donors, transconjugants continued to exist in soil. The imported plasmid may persist in the microbial community if the native transconjugants are effective plasmid donors with a broad spectrum of plasmid hosts and/or high transfer frequency. Inoue et al.'s research was the first to model bacterial conjugation in complex microbial communities.

Sudarshana and Knudsen attempted to mimic plasmid mobilisation between E. coli donors and Pseudomonas fluorescens receivers on pea seeds and roots. They developed a mathematical model to predict mobilisation rates and determine the proportion of triparental

matings that result in plasmid mobilisation. The simple mathematical model was based on the mass action model created by Levin et al. and utilised by Inoue et al. The model assumes that donor and recipient cells are uniformly distributed and that cells grow at the same constant rate. However, despite the fact that these assumptions are not entirely realised in heterogeneous ecosystems like soil, modifications to the mass action model have been successfully utilised to predict bacterial conjugation in soil, the rhizosphere, and the phyllosphere[8].

The most effective way to spread resistance and catabolic genes among bacteria and to get them from other bacteria to adapt to environmental changes is by conjugative plasmid transfer. The mechanism of HGT has been the subject of extensive data collection in recent years; important protein players have been discovered, and their enzymatic processes have been clarified. For plasmids from Gram-negative bacteria, the three-dimensional structure of the protein complexes necessary for horizontal plasmid spread has been solved. Detailed knowledge of the regulatory circuits involved in plasmid transfer of the sex-pheromone-responsive plasmids from Gram-positive enterococci has also been obtained.

CONCLUSIONS

In conclusion, horizontal gene transfer (HGT) is a fundamental process that enables genetic information to be exchanged between microorganisms of the same or different species. This process has important implications for the evolution and adaptation of microorganisms, and has both positive and negative effects. HGT allows for the acquisition of new genes that can confer beneficial traits, such as antibiotic resistance or the ability to degrade specific compounds, which can aid in the survival and adaptation of microorganisms in different environments.

However, HGT can also contribute to the spread of antibiotic resistance and virulence genes, which can pose a significant threat to public health and the environment. Therefore, it is important to study the mechanisms and patterns of HGT to understand how genetic information is transferred and how to mitigate any negative consequences. Moreover, the development of genetic engineering technologies has increased the potential for HGT to be used for biotechnological purposes, such as the production of novel biomolecules or the development of new bioremediation strategies. In summary, HGT is a natural and important process that has both positive and negative consequences. By understanding the mechanisms and patterns of HGT, we can better manage and harness its potential for beneficial applications while minimizing the risks associated with its negative effects.

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CHAPTER 15

POLYMERASE CHAIN REACTION IN MICROBIAL TECHNOLOGY

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ABSTRACT:

Polymerase Chain Reaction (PCR) is a widely used technique in microbial technology for the detection, identification, and quantification of microorganisms. PCR amplifies a specific DNA sequence, enabling the rapid and sensitive detection of target microorganisms in various samples, including environmental, clinical, and food samples. In this review, we provide an overview of the principles and applications of PCR in microbial technology, including the different types of PCR, such as conventional PCR, real-time PCR, and multiplex PCR, and their advantages and limitations. We also discuss the factors influencing PCR performance, such as primer design, template quality, and reaction conditions, and the potential of combining PCR with other techniques, such as DNA sequencing and microbial cultivation, to enhance the accuracy and reliability of microbial detection and identification. Furthermore, we highlight the recent advances in PCR technology, such as digital PCR and loop-mediated isothermal amplification, and their potential to improve the sensitivity and specificity of microbial detection.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

With the advancements in fundamental molecular and biochemical studies, ecology of HGT has kept up. Recent decades have seen tremendous advancements in the study of in situ plasmid transfer, particularly as a result of the collaboration of the fields of molecular biology and microbial ecology. The inner surfaces and organs of soil and water animals, bio-films on a variety of surfaces, and habitats ranging from very oligotrophic to extremely nutrient-rich settings have all been used as experimental evidence for in situ plasmid transfer. All known bacteria seem to use horizontal plasmid transfer as part of their regular lives. This chapter reviews the current understanding of horizontal plasmid transfer in various environments with a focus on polluted habitats. It highlights the role of microorganisms, specifically their mobile me, in the cleanup of polluted environments and the use of MGE in technical bioaugmentation processes to speed up the biodegradation of xenobiotic compounds.

Many whole genome sequences, including those of many indigenous bacteria and many harmful pathogens, have been produced using second-generation sequencing technology. Additionally, because of the frequency of entire plasmid genomes and genomes of other MGE of interest in hospitals and the environment, various research organisations have concentrated on sequencing them. In order to compare various plasmid backbones and better understand how plasmid genome mosaic structures evolved, it will be necessary to determine complete plasmid sequences from various origins with respect to the host background and

habitat. This will eventually make it possible to predict how plasmids will adapt to environmental changes and challenges in the future[1].

The evaluation of plasmid transfer efficiency in complicated settings will be made easier by recent developments in fluorescent reporter technology and ongoing advancements in three-dimensional imaging methods. They will aid in determining plasmid transfer frequencies in nature and, hopefully soon, allow us to forecast HGT events as reactions to environmental stress, along with steadily improving and more accurate models of conjugative plasmid transfer and plasmid mobilisation in complex microbial communities.

Foodborne Bacterial Pathogen Detection Using Molecular Techniques

The traditional approaches of identifying pathogens have frequently relied on diagnosing illness symptoms, isolating and cultivating the organisms, and identifying them using morphological and biochemical assays. The main drawbacks of these culture-based morphological techniques, however, are their time-consuming nature, dependence on the capacity of the organism to be cultivated, and need for significant taxonomic knowledge. Many of these drawbacks may be overcome by using molecular techniques. As a result, throughout the last three decades, there have been tremendous advancements made in the field of molecular identification of bacterial infections. Here, we provide a succinct summary of the molecular detection techniques that may be used to identify food-borne microorganisms.

One of the most pervasive public health issues and a significant contributor to decreased economic output in both rich and developing nations are diseases brought on by tainted food. In the United States, there are around million foodborne illnesses reported year, of which 5,000 need hospitalisation and 5,000 result in death. It is anticipated that between $6.5 and $.9 billion will be spent on medical expenses and lost productivity. Each year, 2.2 million individuals in Canada are thought to get a foodborne disease. In general, it is challenging to identify individual bacterial food pathogens owing to their existence in extremely low quantities among millions of other bacteria. These microorganisms seem to be concealed by the local microflora in the background, and ingredients in the diet may make them harder to find. Additionally, it might be challenging to show that the strains isolated from a food sample are in fact harmful to people. Pathogenic organisms may be quickly and easily detected, which makes it easier to take preventative actions to keep food safe[2].

The inability to separate and cultivate the great majority of bacteria is one of the main obstacles to research on microbial communities and, as a result, the identification of bacteria in the environment. Between the cell counts acquired via direct and viable counts and the counts really taking place in vivo, there is still a discrepancy. Additionally, certain bacteria have been identified as "non-culturable but viable" because they have been found to be uncultivable yet nevertheless remain alive after being exposed to the environment. This characteristic makes it more difficult to identify and count important harmful organisms. A vast range of human pathogens, including Campylobacter spp., Escherichia coli, Listeria monocytogenes, Salmonella and Shigella spp., Vibrio cholerae, V. parahaemolyticus, and V. vulnificus, are documented as entering the VBNC state. Most foodborne bacterial pathogens belong to the genera Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, E. coli, L. monocytogenes, Salmonella spp., Staphylococcus aureus, Shigella spp., and Yersinia enterocolitica.

Conventional culturing methods, including as homogenization, enrichment in nonselective and selective medium, and plating in differential agar media to obtain pure cultures, are still used for the characterisation and detection of foodborne pathogens. Finally, it takes 3–4 days

for phenotypic and genotypic characterisation to corroborate the findings. While DNA-based approaches may be used with mixed cultures or community DNA, biochemical and immunological methods for detection need significant volumes of pure culture. After polymerase chain reaction processes and further sequencing of the amplified product, the final detection stage calls for gel electrophoresis, lengthening the process and adding complexity[3].

Diagnostic labs are currently adopting molecular techniques for frequent pathogen identification. The methodologies available have changed dramatically over the last ten years as a result of developments in molecular biology and biosystematics. Other technologically enhanced approaches, such as second generation PCR and microarrays that provide indefinite multiplexing capabilities, have the potential to raise the efficiency and reliability of pathogen detection to a new and better level.

Molecular Typing Techniques for Bacterial Pathogen Detection

Traditional approaches to pathogen identification have often relied on diagnosing illness symptoms, isolating and cultivating organisms, and identifying pathogens based on their shape and biochemical properties. These culture-based morphological techniques have many fundamental drawbacks, including a dependence on the organism's capacity to be cultivated, the labor-intensiveness of the lab investigations, and the need for considerable taxonomic knowledge. Many of these drawbacks may be overcome by using molecular techniques. Molecular diagnostics and microbiological research have been transformed by DNA-based technologies like the polymerase chain reaction (PCR)[4].

PCR-Based Methods of Detection

The PCR is a method for amplifying certain DNA segments in vitro using two primers. It is often possible to achieve a million-fold amplification of a given area, enabling, among many other purposes, the identification of individual genes in samples. PCR may be used to identify genes involved in the virulence of foodborne bacteria as well as to amplify genes specific to taxonomic categories of bacteria in our lab, a standardised PCR procedure was used for the hly gene amplification.

Nuclease-free water was added to make the final volume. PCR tubes containing reaction mixture were centri- fuged and placed in a thermocycler. Cycling conditions included an initial denatur- ation step at °C for 5 min followed by subsequent cycles consisting of heat denaturation at °C for s, primer annealing examined at °C, °C, and °C, respectively for 1 min, and extension at °C for 1 min. A final extension was per- formed at °C for 5 min to ensure synthesis of all strands. The PCR products were electrophoresed on 1.5% agarose gel which showed a clear band at 4 bp

Several variations of the standard PCR have recently appeared and have contributed to the development of more sensitive detection methods. Be co-amplified in one PCR by combining or “multiplexing” primer pairs. Duffy et al. described PCR-based detection of food- borne pathogens including L. monocytogenes, Salmonella sp., C.jejuni, and E. coli O7:H7. A multiplex PCR protocol was reported for species of foodborne pathogens . Another protocol was reported by Park et al. for the simultaneous detection of E. coli O7:H7, Salmonella spp.,S. aureus, and L. monocytogenes from kimchi.

rPCR allows reactions to be characterized by the time amplification of the PCR product, which can be first detected by the use of a fluorogenic probe. The development of rPCR for the quantification of bacterial load in diverse food matrices has advanced significantly in

recent years. The method's fundamental tenet is the detection of a fluorescent signal that is inversely proportional to the number of amplicons in the sample being evaluated. rPCR-based detection is now commonly employed to identify foodborne bacterial pathogens[5].

PCR has grown in significance as a method for the detection and attribution of microorganisms in recent years. Its popularity is mostly due to the fact that DNA can be amplified from a single bacterial cell in around 1 h, which is substantially quicker than the timeframes required for the earlier approaches. The approach may also increase dead cells, which complicates data interpretation and is a problem that has to be solved since it has long-term repercussions from a legal standpoint. Therefore, while planning experiments, caution must be given. The PCR-based detection procedure for a few foodborne bacterial pathogens has been described in detail by several researchers.

The development of real-time, second-generation PCR has significantly improved diagnostic PCR by removing the need for time-consuming post-PCR sample processing steps and lowering the risk of carryover contamination. Real-time PCR uses closed-tube fluorescence detection and quantification during PCR amplification. Real-time PCR makes it feasible to quantify the quantity of the target pathogen present in the sample in addition to detecting its presence or absence. In order to estimate the likelihood that a disease would develop, it is essential to identify the pathogen as soon as possible. This information also serves as a valuable foundation for making management choices[6].

DISCUSSION

The impact of the food matrix and sample preparation technique on the PCR identification of nonviable cells utilising heat-killed bacteria in ground beef was also studied. Centrifugation, buoyant density centrifugation, immunomagnetic separation, chelex extraction, and swabbing were all used to prepare samples. It was discovered that, as long as the quantity of cells was less than 8 CFU g1, IMS was the only technique that did not provide false-positive findings. This approach's significant shortcoming is that IMS generated false positive results over this threshold.

The first to use the random amplified polymorphic DNA method to analyse anonymous human DNA samples. Several publications have already discussed using the RAPD approach to analyse microbial DNA. The technique doesn't need to know the nucleotide sequence since it employs random primers and works with any species. As a result of this analyses' amplified products' polymorphism, they may be employed as genetic markers. However, the RAPD band does not distinguish between heterozygous and homozygous conditions. The techniques are quite straightforward, and the pieces are scored as dominant Mendelian components. A RAPD methodology was published by Hamza et al. for identifying lactic acid bacteria from traditional Sudanese sour milk. A unique bacterial strain's genome is characterised by the band pattern produced by the analysis. The technique may also discriminate between strains within a species and has the ability to analyse phylogenetic connections among closely related species.

Restriction Polymorphism in Fragment Length

DNA is isolated, digested with restriction endonucleases, size fractionated, transferred from electrophoresis gel matrix to nylon membrane, radiolabeled and chemiluminescent probes prepared, and hybridization to membrane-bound DNA are all steps in the restriction fragment length polymorphism procedure. The probes may be marked with detectable moieties, including enzyme-colorimetric substrates, radioactive isotopes, or chemiluminescent enzymes. The resultant fingerprint is simpler and easier to analyse due to the changes

between species and strains in the position of the restriction enzyme sites and with the specificity of the probe. Compared to other probes that are more species- or strain-specific, the rRNA probe is more appropriate for a broad range of bacteria. Ribotyping is the process of using this probe for characterisation, which combines Southern blot hybridization with restriction enzyme digestion for analysis. The probe may be one of the rRNA genes or a combination of the rRNA genes and the spacer sequences since the ribosomal operons in bacteria are divided into S, S, and 5S rRNA and are often separated by noncoding spacer DNA. Depending on the utilised probe, hybridization patterns vary. For ribo-typing, labelled probes comprising the S, S, and 5S rRNA sequences of E. coli are most often utilised.

It has been shown that ribotyping is useful for identifying strains, such as Carnobacterium species, that are challenging to type using conventional phenotypic techniques. For four closely related Carnobacterium species that are valuable to the food business, Kabadjova et al. developed a quick PCR-RFLP-based identification method. Three isolates that had been misclassified as C. divergens were reclassified as C. piscicola using the fast PCR-RFLP technique. Similar to how four isolates previously categorised as C. piscicola were reclassified as C. divergens based on the S-S ISR-RFLP techniques' patterns. A group at Keygene N.V. in Wageningen, The Netherlands, under the direction of Marc Zabeau, created amplified fragment length polymorphism analysis. The basic idea behind the AFLP fingerprinting method was explained by Vos et al. A version of RAPD called AFLP uses PCR amplification to identify restriction fragments in order to find restriction site polymorphisms without previous sequence information[7].

In essence, AFLP is a method of genome fingerprinting based on the PCR amplification of just a few fragments obtained through restriction digestion of the whole genome. Two restriction enzymes are used in the fundamental technique to break down enzymes, producing DNA fragments with two distinct kinds of sticky ends. Adapters are ligated to create PCR templates in order to achieve these goals. Two separate primers that have the same sequence as the adapters but have been expanded to add one or more selective bases close to the restriction site of the primer are used to carry out the selective amplification process. Only pieces that completely match other pieces are magnified. About - DNA fragments are obtained with this method, some of which are strain- and species-specific.

One of the most reliable multiple-locus fingerprinting approaches for genetic markers that has been tested for genotypic characterisation is AFLP analysis. The genetic links between Xanthomonas axonopodis pv. Manihotis strains were characterised by Restrepo et al. using AFLP. Through comparison of freshly acquired data with findings previously obtained by well-established genotypic and chemotaxonomic techniques such DNA hybridization and cellular fatty acid analysis, Janssen et al.'s work demonstrated wide applicability of AFLP in bacterial taxonomy.

Electrophoresis using Pulsed-Field Gel

The pulsed-field gel electrophoresis technique was developed by Schwartz and Cantor as a way to generate a molecular karyotype from the yeast Saccharomyces cerevisiae's chromosomal DNA. The foundation of PFGE is the digestion of chromosomal DNA using specialised cutting enzymes. The overall number of DNA fragments is reduced by the employment of these enzymes.

By using alternately pulsed electric fields that are produced perpendicular to each other and of which one is inhomogeneous, this technique may separate big DNA molecules. Using repeated alternating electric fields to enable the DNA molecules to continually shift their migratory orientation is the fundamental idea behind PFGE. In order to fit through an agarose

pore opening, the big DNA molecule must uncoil and extend parallel to an electric field. The molecule must reorient itself to enter a new opening when the electric field is switched off and a fresh electric field is introduced perpendicular to the opened DNA. To better separate DNA fragments of various sizes, the pulse period and electron force are continually increased. Arbeit claims that PFGE is more accurate and selective than several other techniques for identifying microorganisms. The technique has been researched for application in epidemiological investigations, including those involving Campylobacter, since it is able to differentiate between the species and strains implicated in foodborne outbreaks.

Due to its selective powers, PFGE became the accepted method for bacterial foodborne illness epidemic analysis in. To ensure repeatability across laboratories, uniform protocols have been created for PFGE procedures and data interpretation. PFGE is thus regarded as the "gold standard" for molecular-based research. It has evolved into the go-to subtyping technique for networks developed in the US and Europe for monitoring and the gathering of PFGE fingerprints of bacteria linked to foodborne illnesses. As of now, S. enterica sv. Braenderup strain H digested with XbaI and PFGE procedures for Shiga toxigenic E. coli O7, S. enterica, Shigella spp., L. monocytogenes, thermotolerant Campylobacter spp., and V. cholerae have been standardised by PulseNet USA. Biosensors are devices or instruments that include a biological sensing component connected to a transducer. In addition to enzymes, organelles, antibodies, entire cells, DNA, and tissue, biological sensing components may also incorporate them. Electrochemical, calorimetric, optical, acoustical, or mechanical transducers are some examples of transducers. Electrochemical DNA biosensors with the ability to detect nucleic acids sensitively and specifically based on their sequence may now be created thanks to microfabrication technology. Electrochemical sensors have a clear advantage over methods like PCR that need on target purification and amplification since they can detect nucleic acids directly in complicated mixtures. Recognition of pathogen-specific signature sequences in bodily fluids may be possible by using DNA sensor technologies to study infectious illnesses.

The most important stage in creating an electrochemical biosensor is immobilising a DNA probe on the chosen substrate since it has a substantial impact on the sensor's sensitivity, specificity, and repeatability. The terminal of the DNA molecule or the substrate's surface must be functionalized for DNA to attach to its substrate in an efficient manner. DNA probe immobilisation has been effectively accomplished using the affinity of streptavidin and biotin. Due to their covalent attachment to thiolated DNA, gold substrates are also attracting a lot of research. Rapid, portable, and affordable testing methods are of particular importance to this technology. E. coli:H7 DNA paired with PCR has been detected effectively using electrochemical biosensors. The quick detection of food pathogens has been accomplished using a biosensor and gold nanoppapers[8].

For the detection of certain DNA sequences, gold ppapers with a diameter of a few nanometers have been utilised. A method to employ nonfunctionalized GNP for the detection of dsDNA and ssDNA was developed since functionalized chem- istry is not widely used due to the expenses involved. In this technique, the colloidal condition of citrate-coated GNPs is characterised by a distinctive red hue. The addition of salts may easily trigger the aggregation of GNPs, producing a purple colour. The unassisted eye can see the difference in colour. The hydrophilic, negatively charged phosphate backbone of ssDNA, which interacts electrostatically with the negatively charged GNP surface through Vander Waals interactions, may uncoil and become exposed to aqueous solution. These interactions give GNPs a negative charge and make them more repellent. These characteristics have been used to create a biosensor that can quickly identify a PCR result directly in the same tube.

An electrochemical sensor assay for the detection of bacterial pathogens such as E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aero- genes, Enterobacter cloacae, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter freundii, and Enterococcus faecalis has been developed using microfluidics-based " The presence of bacteria was determined using an evanescent wave fibre optic biosensor on samples of ground beef. The biosensor creates the evanescent wave by shining light onto optical fibre probes using a 5-nm laser diode. A fraction of the emission recouples into the fibre probe after fluorescent molecules in the evanescent field are activated. The fluorescent signal is recognised and quantified using a photodiode. A sandwich immunoassay was utilized, which allowed the detection of 9.0 × 3 CFU g−1 for g samples and 5.2 × 2 CFU g−1 for the -g sample. No false positives were obtained with results obtained min after sample processing.

CONCLUSION

The Polymerase Chain Reaction (PCR) has revolutionized the field of microbial technology by allowing for the rapid and accurate amplification of DNA sequences. This technique has numerous applications in microbial ecology, biotechnology, and medical diagnostics. PCR has enabled the identification and characterization of microbial communities and individual microorganisms by targeting specific genes or sequences of interest. This has allowed for a better understanding of microbial diversity and function in various environments. Moreover, PCR has facilitated the development of new biotechnological applications, such as the production of recombinant proteins, the engineering of microbial genomes, and the development of new medical diagnostics and therapeutics. However, PCR has limitations, such as the potential for contamination and the possibility of amplifying non-target DNA sequences.

These limitations can be mitigated by implementing appropriate controls and utilizing advanced PCR technologies, such as real-time PCR and digital PCR. PCR is a powerful tool in microbial technology that has numerous applications and has significantly contributed to our understanding of microbial diversity and function. With continued advancements in PCR technology and methodology, this technique will continue to play a critical role in microbial research, biotechnology, and medical diagnostics.

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CHAPTER 16

A BRIEF DISCUSSION ON MICROARRAYS TECHNIQUE

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ABSTRACT:

Microarray technology is a powerful tool for high-throughput analysis of gene expression, genotyping, and detection of microbial pathogens. Microarrays are essentially miniaturized platforms that allow for the simultaneous analysis of thousands of genes or nucleic acid sequences in a single experiment. This review provides an overview of the principles and applications of microarray technology in microbial research, including the different types of microarrays, such as DNA microarrays, protein microarrays, and tissue microarrays, and their advantages and limitations. We also discuss the steps involved in microarray analysis, including sample preparation, probe design, hybridization, and data analysis. Furthermore, we highlight the potential of microarray technology in various fields, such as microbial ecology, clinical microbiology, and biotechnology. Additionally, we discuss the challenges and future prospects of microarray technology, emphasizing the need for the development of more sensitive and specific microarray-based assays for microbial detection and monitoring.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Single nucleotide polymorphism profiles and gene expression research were the initial goals of the DNA microarray technique. It is now used to diagnose newly developing infections. The platform provided by microarray technology allows for infinite multiplexing. Using microarray technology, thousands of distinct DNA or RNA sequences may be found at once on a tiny glass or silica slide of around 1-2 cm2.DNA microarrays are solid surfaces to which several probes, DNA fragments, or oligonucleotides are immobilised in order to hybridise with target DNA from the sample that has been fluorescently labelled. The target might be an amplified PCR product or genomic DNA extracted from the sample. Genomic microarrays and oligonucleotide arrays are the two main categories of DNA microarrays. While in oligonucleotide microarrays the target DNA hybridises nucleotide-length oligonucleotides, whole genes or their fragments from a strain of a microorganism serve as the probes in genomic DNA microarrays. Despite the fact that any form of microarray may be utilised for pathogen detection, oligonucleotide microarrays are often preferred for the detection of either genomic DNA directly or the PCR-amplified component of the genomic DNA, such as rRNA genes or virulence genes[1].

For the purpose of identifying Bacillus spp., C. difficile, and other foodborne bacterial infections, microarrays have been created. juni, E. coli, L. monocytogenes,S. Vibrio species, enterica, Shigella dysenteriae, and Staphylococcus species. in the event of food poisoning outbreaks and biological warfare, for the discriminating of numerous pathogens and their virulence factors. The employed comparative genomics to choose mer oligonucleotide probes

specific for key foodborne pathogens to be used in microarray analysis in order to build a technique for the precise detection and identification of food-borne illnesses. Researchers looked at how the genomic DNA of diverse bacteria and foodborne diseases hybridised with this specially designed microarray. With each pathogen's genomic DNA, a very specific hybridization pattern was seen. The GenePix Pro 6.0 and GeneSpring GX programmes were used to analyse and cluster the microarray data. The developed microarray's ability to discriminate was shown by the dendrogram. Each foodborne pathogen was grouped according to the specificity of its hybridization, and pathogenic and nonpathogenic species were separated. This technique may be used in the food sector for the quick and precise detection and identification of foodborne microorganisms. In addition, epidemiological and taxonomic research, as well as the domains of food safety and biodefence, may benefit greatly from genome sequence comparison and DNA microarray analysis.

Comprehensive Systems

Some integrated systems have developed during the last few years, and some have even been reported for the identification of bacterial infections. These techniques are well-liked because they shorten analysis durations and boost detection effectiveness. Lu et al. recently created an on-chip immunoassay that recognises the intracellular antigen of L. Using the Aad antibody, monocytogenes based on polystyrene beads were functional. In the microfluidics channel, polystyrene beads were extensively combined with cell lysate to bind the beads to the antigen in the lysate. The beads were exposed to fluorescently labelled Aad, and after washing, the fluorescence output from the beads was inversely related to the amount of bacteria that could be seen. When used with cell lysate-based immunoassays, this chip may be helpful. A microfabricated DNA analysis device with integrated PCR and capillary electrophoresis was disclosed by Woolley et al. The method combines heat cycling with CE chips' quick DNA separation. Within a minute, our technology generated a genomic DNA test for Salmonella. A micromachined chemical amplifier was utilised by Andreas Manz's team to quickly and continuously run PCR. According to the authors, amplification is independent of input concentration and DNA input and output are both continuous. Neisseria gonorrhoeae was examined, and a -cycle PCR was finished in s to.7 min, depending on flow rate, according to their report[2].

The Lawrence Livermore National Laboratory has developed an enhanced nucleic acid analyzer for the identification of bacterial pathogens such Erwinia herbicola, Bacillus subtilis, and B. the anthrax. Ten silicon reaction chambers with thin-film resistive heaters and solid-state optics made up the apparatus. According to the scientists, detection periods might be as little as a minute and there could be as many as 2-4 organisms per millilitre. The instrument's absence of moving components makes it durable and enables quick analysis, minimal power consumption, and real-time monitoring.

The goal of this review paper was to demonstrate the variety of novel approaches that may be used to identify bacteria in food samples rather than to provide a comprehensive list of all the species that have been discovered using molecular techniques. Identification of foodborne pathogens is crucial to providing for people's health. for compared to molecular approaches, biochemical and immunological methods need more time and have lower sensitivity for isolating and identifying foodborne microorganisms. Using DNA polymorphism among various bacterial species, food pathogens have been identified.

These bacterial pathogens may now be identified using PCR techniques. PCR is an efficient, quick, trustworthy, and sensitive method for detecting the genes of bacterial pathogens in a variety of foods. The 5 nuclease multiplex PCR technique has also been used to

simultaneously screen for bacterial pathogens in diverse environmental materials and food products. The technique will also work for microorganisms that are slow-growing or uncultivable. The procedures based on electrophoresis that are discussed in this chapter take a lot of time and effort. For the differentiation of bacteria at the species level, RFLP needs pure culture. The RAPD approach has the drawbacks that repeatable amplification results need standardisation of primer concentration and tem-plates, and the majority of RAPD markers are dominant, making it difficult to discriminate between amplified DNA sequences with comparable DNA sequences. The partial digestion of chromosomal DNA, which may produce an abnormal AFLP pattern, is a challenge in AFLP analysis. The "gold standard" for detecting the pathogenic microorganisms in situations of food poisoning, water contamination, and hospital epidemics has been PFGE. Due to its selective powers, PFGE has evolved into the industry standard for analysing bacterial foodborne illness outbreaks. Despite being dependable and accurate, the approach is sluggish since sample preparation and analysis take a long time. Real-time PCR enables more quick data capture with less modification. Real-time amplification monitoring now makes it unnecessary to do time-consuming postamplification processing processes like gel electrophoresis. Real-time PCR provides higher multiplexing opportunities, however multiplexing is still restricted by the availability of dyes generating fluorescence at various wavelengths. Therefore, it is presently not feasible to identify more than a few diseases using these techniques.

Theoretically, the microarray technology, a recent and developing pathogen testing tool, provides a foundation for limitless multiplexing capabilities. On the glass slide that forms the chip, tens of thousands of these probes may be seen in a specific and addressable configuration. Microarrays hold great potential for the identification of all pertinent pathogens inside a particular food matrix due to their limitless capacity for simultaneous pathogen detection. The creation of microarrays for diagnostic applications in food microbiology is a new development in this discipline that has been covered in length in this chapter. Although microarrays have made analyses more expeditious, there are disadvantages to their usage. The enormous quantity of data produced by microarray devices must be sorted through using specialised expertise and training, which is costly and difficult to get. This restricts the scope of microarray technology's use in typical labs. More work is required to overcome the difficulties of examining food samples where pollutants such organic compounds and heavy metals may interfere with DNA hybridization and influence the effectiveness of microarrays. This endeavour to provide a quantitative component to microarrays must continue.

Microbial biosensors and bioassays have so far been used more for the direct monitoring of food pathogens than for the detection of food additives and pollutants. Few biosensors are now on the market, despite the fact that research on them has sometimes emerged in the literature during the last two decades. The sensitive nature of the biological component and the miniaturisation of the electrical components are significant limitations. Reliable biosensors for the online detection of biological events will be available in the future as electrical innovation continues to produce smaller and more dependable electronic devices and as biological sciences continue to expand their unique knowledge of enzyme and microbial genetics. The quick identification of microbes is one discovery that will greatly assist the food business.

Although the techniques above are very precise and specific, the greatest care must be taken to standardise procedures for isolating DNA from microorganisms in food samples. Additionally present is the DNA of extinct bacteria, which may amplify and provide erroneous positive findings. To differentiate between dead and alive microorganisms, use

ethidium monoazide. BDC, sometimes known as flotation, may be used to effectively remove free DNA from samples before analysis. By avoiding DNA from VNBC bacteria, this may reduce the possibility of false-positive findings. Recent Advances in Bioremediation of Contaminated Soil and Water Using Microbial Surfactants[3]

It is well recognised across the globe that inappropriate disposal of industrial, mining, agricultural, municipal, and other leftovers may cause environmental pollution. The removal of such contaminants from soil and water is now being accomplished using a variety of chemical, physical, and biological-based techniques. Biological therapy, or remediation employing bacteria, is one of these treatments and one of the most promising due to its affordability and almost total eradication of many contaminants. The capacity of the deteriorating microorganisms to survive throughout the procedure is a key prerequisite for this approach. When contaminants are not easily bioavailable, biosurfactants, especially microbial surfactants, are crucial because they increase the apparent water solubility of the pollutants. This may be done by either ex situ addition or in situ generation of biosurfactants by microorganisms. However, since microbial surfactants have such a broad range of potential applications in the environmental field, it's critical to understand how they work, new developments in bioremediation techniques, and other potential uses. Therefore, the objective of this chapter is to provide an overview of the many kinds of microbial surfactants and sources, their functions in various bioremediation procedures, and current developments in the area.

As the world's population, industry, and urbanisation rise, so does the amount of harmful heavy metals, polycyclic aromatic hydrocarbons, petrochemicals, pesticides, and herbicides that pollute soil and water. If these toxins are discharged into the environment without being properly treated, they may cause soil and water pollution in industry, mining, and agriculture. Environmental issues might also result from accidental spills. Treatment of polluted soil and water has grown to be a significant public health concern globally due to the possible toxicity, mutagenicity, and carcinogenicity of these pollutants. There are a number of traditional physical and chemical procedures that may be used to remediate polluted areas, but their main drawbacks include their high cost and inability to totally remove contaminants. By using microorganisms or their products, "bioremediation" refers to the removal of polluted soil and water. This method has shown tremendous promise in terms of cost-effectiveness and the capacity to totally destroy numerous contaminants[4].

A competent microbial culture that can last in polluted medium and thoroughly and effectively destroy pollutants is a need for this approach. Either in situ or ex situ bioremediation is possible, although the former is preferable. In situ bioremediation often entails the direct introduction of microorganisms to the polluted site; these organisms use pollutants as their only carbon source or cometabolize them with other carbon sources. The presence of pollutants that are mostly insoluble in water restricts the development of microbes, and biosurfactants, in particular microbial surfactants, are crucial for bioremediation because they make toxins seem more soluble in water. Microbial surfactants are widely used in the environmental field, thus it's crucial to understand how they work, new developments in bioremediation methods, and other potential applications. In this chapter, the many kinds of microbial surfactants and their sources, as well as their functions in various bioremediation procedures and new developments in the area, are briefly discussed.

When cultivated on water-miscible or oil-based substrates, a variety of microorganisms create microbial surfactants, also known as biosurfactants. Biosurfactants are created by bacteria, yeast, and fungus; as a result, they have a wide range of structural characteristics and may be categorised according to these differences. Glycolipids. The most frequent and extensively

researched biosurfactants are glycolipids. They have a lipid tail and a carbohydrate head, and they are nonionic. Among glycolipid-type biosurfactants, rhamnolipids, sophorolipids, and trehalose lipids are the most well-known. Rhamnose sugar molecules one or two coupled to molecules of -hydroxydecanoic acid make up the structural core of rhamnolipids. The production of this particular class of biosurfactants by a few Pseudomonas species was initially noted by Jarvis and Johnson.A few yeasts of the Candida species primarily create sophorolipids, which are made up of a dimeric glucose connected by a glycosidic bond via a hydroxyl group at the penultimate position of a -carbon fatty acid. This kind of biosurfactant may be acetylated at the main hydroxyl positions of the sophorose sugars and is found in a combination of macrolactone and open-chain forms. Using the yeast Torulopsis magnoliae, Gorin et al. were the first to describe extracellular sophoro-lipids. Sophorolipids are a kind of biosurfactant that may be generated by non-pathogenic Candida species in considerably higher concentrations than other biosurfactants like rhamnolipids, which are created by pathogenic microbes. When it comes to trehalose lipids, disaccharide trehalose sugar, which is mostly generated by Mycobacterium, is connected to mycolic acid.

Arthrobacter, Nocardia, Corynebacterium, and Rhodococcus species both lipoproteins and lipopeptides. Microorganisms create lipopeptides and lipoproteins, which are recognised more for their antibiotic action than for their surfactant properties. The most extensively researched biosurfactant in this category is surfactin, which is generated by the bacterium Bacillus subtilis and was initially discovered by Arima et al. phospholipids, neutral lipids, and fatty acids. When cultivated on n-alkanes, a number of bacteria create considerable amounts of fatty acids, phospholipids, or neutral lipids and are regarded as biosurfactants. Simple straight-chain fatty acids generated by bacteria may also be complicated in form and comprise OH groups and alkyl branching. Long-chain, -hydroxy fatty acids replaced at the end of mycolic acids

Mycobacterium, Nocardia, Rhodococcus, and Corynebacterium species are the main producers of these compounds, which have a -carbon atom and a somewhat long aliphatic chain. Another instance of a complex fatty acid biosurfactant produced by Rhodococcus erythropolis is corynomucolic acid. All eukaryotic cells contain triacylglycerols, and the bacterium Acinetobatcer sp. produces esters. Examples of neutral lipids include certain eukaryotic algae and related bacteria like Moraxella. Aspergillus sp. When cultivated on n-alkanes, strain HO1-N generates phosphatidylethanolamine, a phospholipid surfactant. When Aspergillus sp. Additionally, it has been shown that Thiobacillus thiooxidans produces significant amounts of phos- pholipids [5].

Important Biosurfactant Qualities

Biosurfactants have many characteristics with chemical surfactants. The most significant characteristics are covered here. Critical micelle concentration and the lowering of surface or interfacial tension are two criteria used to assess surfactants. Molecules of biosurfactant have a tendency to bond with one another or with surfaces between phases of varying polarity. The most obvious result of their influence on these interfaces is a decrease in surface tension. Standard techniques may be used to quantitatively quantify the surface tension at the air/water and oil/water interfaces. A threshold is eventually achieved when the surface tension of the medium takes on a minimum value and the surfactant monomer starts to form micelles as the surfactant concentration is increased in an aqueous medium. The CMC is the level of surfactant concentration at which micelles start to form. Surface tension is constant above the CMC, suggesting that micelle formation has occurred in the bulk phase and the interface is saturated. A lower CMC means that less surfactant is needed to permeate the air-

liquid or liquid-liquid interface to create micelles. As a result, CMC is the most widely utilised indicator of surfactant effectiveness[6].

Biosurfactants are especially well suited for bioremediation since they are biodegradable, one of their main benefits over synthetic surfactants. Pseudomonas aeruginosa, B., and Zeng investigated the codegradation of artificial surfactants and rhamnolipids with glucose. microorganisms that compost and subtilis in liquid culture medium. In addition to being resistant to microbial destruction, CTAB prevented organisms from using the media's easily accessible carbon supply. Triton X-0, a nonionic surfactant, proved resistant to biodegradation while being harmless to microorganisms and hence not inhibiting growth. SDS, an anionic surfactant, might be codegraded with glucose as a carbon source while being nontoxic to bacteria. The biosurfactant rhamnolipid was a special kind of non-toxic, B-degradable surfactant. It may be used by P. aeruginosa, the organism that produced it, but not by B. subtilis or compost microbes. According to studies done by several authors, the sophorolipids generated by Candida bombicola are quickly biodegradable biosurfactants.

Small Toxicity

When compared to chemical surfactants, microbial surfactants are often thought of as less hazardous or nontoxic compounds and may thus be more suitable for medicinal, cosmetic, and food uses. Poremba et al. discovered that a chemically generated anionic surfactant had an LC against Photobacterium phosphoreum that was ten times lower than rhamnolipids, indicating a greater level of toxicity. Sophorolipids also showed negligible cytotoxicity towards human fibroblasts, the HPK II cell line of human keratinocytes, and regular human epidermal keratinocytes.

Using Various Physical, Chemical, and Biological Techniques to Clean Up Contaminated Soil and Water

Hazardous chemical contamination of soil and water is a serious global public health and environmental issue. In general, sectors including petrochemicals, pulp and paper, chemical manufacture, mining, and others produce hazardous compounds as byproducts. Researchers have successfully used a number of physical, chemical, and biological remediation techniques, but more frequently than not, a single remediation strategy may not be sufficient to remove contaminants from soil or water; as a result, combinations of two or more remediation techniques are frequently used for achieving the best results. Below are some brief explanations of various methods[7].

DISCUSSION

Physical Techniques

Separating the pollutants from soil or water is the goal of physical remediation approaches. Thus eliminated contaminants are often treated ex situ. Among physical procedures, excavation and soil vapour extraction are most often utilised. Other physical methods for treating polluted soil and water include air sparging and soil washing with surfactant or cosolvent flushing. Typically, these procedures are expensive and need additional care.Chemical Methods

For the remediation of polluted soil and water, oxidising chemicals such H2O2, Fenton's reagent, permanganate, ozone, and sodium persulfate are often utilised. The kind of oxidant used will depend on the nature and amount of contamination, the feasibility of oxidant delivery, the soil conditions, and the hydrogeology of the location. The capacity to remediate pollutants that are present in high concentrations and the relatively quick treatment time are

the main benefits of chemical approaches. One of the main drawbacks of these approaches is their nonselectivity. Another is their capital expense. Additionally, a lot of oxidising agents have a high degree of reactivity, which causes high reaction temperatures and the possibility of an explosion. The use of these drugs requires that field scientists have the necessary training.

Techniques Based on Biology or Bioremediation

A new and effective method for treating polluted soil and water is bioremediation. The main prerequisites for bioremediation are that the microorganisms utilised must be effective at decomposing the pollutant and able to survive in the polluted environment. As a result, bioremediation is only successful in environments that support microbial development and activity. When necessary, environmental factors must be changed to promote microbial growth and pollutant breakdown. Bioremediation may be aerobic or anaerobic, depending on the organism and the harmful pollutant. Several microbial species, including Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium, have been linked to the aerobic degradation of pollutants such pesticides and hydrocarbons. Many of these bacteria rely entirely on the contamination for their carbon and energy needs. The primary benefits of this method are its low cost and permanent elimination of the pollutant by full mineralization of the contaminant. Additionally, the method may be created to be non-invasive and preserve the ecosystem. In situations when physical or chemical techniques of treatment would be impractical, bioremediation may handle low quantities of pollutants. Longer process timeframes and limited system predictability are this technique's main disadvantages as compared to traditional approaches. The following is a description of a few bioremediation techniques.

This approach is often the best choice since it is less expensive, causes less disruption, and treats toxins where they are, without the need for excavation and transportation. The depth of the contaminated soil that may be treated limits in situ therapy. Although depths of cm and higher have sometimes been successfully treated, efficient oxygen diffusion for desirable rates of bioremediation typically only extends to a few centimetres to around cm in many soils. By dissolving air, peroxide, ozone, and other chemicals in influent water, oxygen may be pushed to lower depths to solve this problem. The key in situ land treatments are briefly explained in more detail.

The most popular in situ remedy is called "bioventing," which involves injecting air and nutrients into polluted soil via wells to encourage the growth of local microorganisms. While waste vapours are being removed by negative pressure, these organisms will attempt to breakdown the pollutants. Low air flow rates and just the quantity of oxygen required for biodegradation are used in bioventing to reduce volatilization and the release of contaminants into the atmosphere. It may be used if pollution is found far below the surface and is effective for simple hydrocarbons[8].

Biosparging: In this in situ method, air is introduced below the water table to raise the oxygen content of groundwater and speed up the pace at which bacteria degrade pollutants biologically. With this approach, mixing is increased the saturated zone, enabling interaction between groundwater and oxygen in the soil. Small-diameter air injection points are simple and inexpensive to install, which gives designers and builders of the system a great deal of freedom.

Bioaugmentation: This in situ method typically entails adding microbes, either exogenous or native, to the polluted locations. The use of additional microbial cultures is constrained by two factors: first, most soils exposed to biodegradable waste have native microorganisms that

are efficient degraders; second, nonindigenous cultures rarely compete with an indigenous population well enough to develop and sustain useful population levels.

Ex situ techniques entail digging up or removing contaminated soil. After that, the soil receives surface-level treatments. Landfarming is a straightforward procedure in which polluted soil is dug, deposited on a bed that has been prepared, and repeatedly tilled until pollutants are broken down. The goal is to promote aerobic breakdown of pollutants and to activate native microorganisms. The practise is often restricted to treating the top soil layers. Landfarming has drawn a lot of interest as a remediation option since it has the potential to lower monitoring and maintenance expenses as well as clean-up obligations.

Composting is the process of mixing polluted soil with organic materials that aren't harmful, such manure or agricultural waste. These organic ingredients encourage the growth of a diverse microbial consortia and high temperatures. By using this method, hydrocarbon pollutants are broken down chemically and biologically. Biopiles Fahnestock et al. combine composting with land cultivation. Engineered cells are made to resemble aerated compost heaps and are used to remediate petroleum hydrocarbon surface pollution. This is a more sophisticated kind of land farming that has the tendency to reduce pollutant losses via leaching and volatilization. The activity of local aerobic and anaerobic bacteria is made more favourable by biopiles.

Bioreactors. For the ex situ treatment of polluted soil and water, slurry reactors or aqueous reactors are utilised. A slurry bioreactor is a device used to boost the bioremediation rate of soil-bound and water-soluble contaminants by establishing a three-phase mixing state. The breakdown rate of contaminants in bioreactors is high due to the establishment of optimal environmental conditions and high mass transfer rates. However, the bioreactor system must first be physically installed in the soil. Reactor conditions must also be carefully observed.

Biosurfactants are crucial in the bioremediation of soil and water polluted with hydrophobic contaminants as well as in the removal of heavy metals from contaminated soil due to their capacity to diminish surface/interfacial tension between two phases and form micelles. The following discussion covers the function and mechanism of biosurfactants in the bioremediation of locations affected with different contaminants. Organic substances that only contain hydrogen and carbon are known as hydrocarbons.

They may be divided into four groups: aromatic hydrocarbons, cycloalkanes, saturated hydrocarbons, and unsaturated hydrocarbons. Although they are largely employed as energy sources, their mutagenicity, carcinogenicity, and propensity to bioaccumulate in the food chain make them harmful to the environment and human health if found in soil or water.

CONCLUSION

Microarrays are a powerful and versatile technique that has revolutionized the field of microbial technology by enabling the simultaneous detection and analysis of thousands of genes and sequences. This technique has numerous applications in microbial ecology, biotechnology, and medical diagnostics. Microarrays have enabled the identification and characterization of microbial communities and individual microorganisms by analyzing the expression of multiple genes or sequences simultaneously. This has allowed for a better understanding of microbial diversity and function in various environments. Moreover, microarrays have facilitated the development of new biotechnological applications, such as the production of recombinant proteins, the engineering of microbial genomes, and the development of new medical diagnostics and therapeutics. However, microarrays have limitations, such as the potential for false positives and the need for specialized equipment

and expertise. These limitations can be mitigated by implementing appropriate controls and utilizing advanced microarray technologies, such as next-generation sequencing-based microarrays.

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CHAPTER 17

A STUDY ON POLYCYCLIC AROMATIC HYDROCARBONS

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ABSTRACT:

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that result from the incomplete combustion of organic materials. PAHs are harmful to human health and the environment due to their carcinogenic, mutagenic, and teratogenic properties. Microorganisms play a vital role in the biodegradation of PAHs, and various microbial species have been identified that can metabolize these compounds under different environmental conditions. This review provides an overview of the biodegradation of PAHs by microorganisms, including the mechanisms involved in PAH degradation and the factors that influence microbial PAH degradation, such as temperature, pH, oxygen availability, and nutrient availability. We also discuss the applications of microbial PAH biodegradation, such as bioremediation of contaminated soils and waters, and the challenges and limitations associated with this process, including the potential for the formation of toxic intermediate metabolites and the need for the development of more effective and sustainable bioremediation strategies.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

The PAH class of organic compounds has two or more fused aromatic rings. Naphtalene, phenanthrene, anthracene, and pyrene are a few examples of PAHs. Numerous PAHs have been demonstrated to have cancer-causing abilities. Their release and subsequent accumulation in terrestrial environments have sparked concern. It is necessary to clean up soil that contains these substances. Due to PAHs' limited water solubility, which increases their sorption to surfaces and lowers their bioavailability to microorganisms, it is challenging to bioremediate sites that have been contaminated with these compounds. Biosurfactants may encourage microbial growth on bonded substrates by eliminating biosurfactants from surfaces or by increasing their perceived water solubility. Surfactants that considerably lower interfacial tension are particularly effective in liberating bound hydrophobic molecules and making them available for biodegradation[1].

Low-molecular-weight biosurfactants boost the apparent solubility of hydrocarbons by integrating them into the hydrophobic chambers of micelles. Numerous authors have looked at how different biosurfactants could quicken PAHs' biodegradation. Once within the cell, the necessary enzymes start attacking and destroying the PAH. Another putative biosurfactant action mechanism involves altering the hydrophobicity of cells. It will be simpler for cells to directly interact with PAHs if a biosurfactant sticks to the cell membrane and modifies the cell's hydrophobicity, which encourages PAH absorption and biodegradation. Alasan produced by Acinetobacter radioresistens KA increases the apparent aqueous solubilities of

phenanthrene, fluoranthene, and pyrene by up to 6.6,.7, and.8-folds, respectively. Rhamnolipid biosurfactant increases the apparent aqueous solubility of phenanthrene and total degradation when combined with salicylate or glucose in a liquid solution by at least% as compared to solutions that also included salicylate or glucose without biosurfactant.

The elimination of PAHs was shown to be 40-50% more effective when rhamnolipid was added, according to a recent research by Sponza and Gok. They conducted a continuous stirred tank reactor evaluation of the PAH degradation in a petrochemical effluent. The breakdown of five- and six-ring PAHs was markedly accelerated by rhamnolipid treatment. Arun et al. created a biosurfactant-producing Pseudomonas strain from oil-contaminated soil and found that it lowered% of pyrene. Table 9.3 summarises the research on the microorganisms' ability to break down different hydrocarbons with the use of biosurfactants. When n is the number of hydrogen atoms and 2n is the number of carbon atoms, an aliphatic molecule is referred to as a hydrocarbon. Low-molecular-weight alkanes are the petroleum hydrocarbons that bacteria break down most rapidly. As the chemical's chain length and branching grow, it becomes more resilient to microbial attack[2].

According to research, biosurfactants speed up the biodegradation of petroleum hydrocarbons via PAH-like mechanisms. Zhang and Miller demonstrated that rhamnolipids at a concentration of 0 mg/l increased octadecane mineralization by 5 to % compared to controls. Rahman et al. studied n-alkane bioremediation in a petroleum sludge with a.4% oil and grease content. The following degradation efficiencies in% sludge were noted after days after the addition of a bacterial consortium, nutrients, and rhamnolipids: C8-C alkanes: 0%; C-C: -%; C-C: -%, and C-C: -%. The significance of adding rhamnolipids in speeding the biodegradation of even extremely hydrophobic molecules was shown by the fact that, despite the rate of biodegradation decreasing as the chain length rose, rates were still significantly high even for C-C compounds. Whang et al. investigated the potential use of two biosurfactants, surfactin and rhamnolipid, for faster biodegradation of diesel-contaminated soil and water in a series of bench-scale studies.

Adding mg/l of surfactin significantly boosted the biodegradation of the diesel up to% in batch testing using diesel and water as compared to% when no surfactin was added. Similar to this, adding rhamnolipid to diesel/water systems in the range of 0- mg/l greatly improved the biodegradation of diesel from to 0%. These results confirmed the biosurfactant's potential for speeding up and enhancing the biodegradation of diesel in both soil and water systems. In contrast to synthetic surfactants, Lai et al. looked at the ability of two biosurfactants to break down total petroleum hydrocarbons from soil. The researchers asserted that biosurfactants significantly outperformed synthetic surfactants in eliminating TPH. By using 0.2% of rhamnolipids, surfactin, Tween, and Triton X-0, respectively, TPH was removed from soil that had been contaminated with roughly 3,0 mg of TPH/kg of dry soil. Thanks to the surfactants, the removal efficiency for soil contaminated with about 9,0 mg TPH/kg soil increased to,,, and%, respectively. TPH removal efficiency increased along with biosurfactant concentration, but did not significantly alter when contact duration was extended from 1 to 7 days.

Using Pesticides and Herbicides

The insecticides dichlorodiphenyltrichloroethane, 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, plasticizers, pentachlorophenol, and polychlorinated biphenyls are a few examples of halogenated aromatic compounds. Due to their stability and toxicity, the environment and general public health are at danger. The halogenated aliphatic molecule, the location, and the amount of halogens all affect the pace and process of biodegradation. It

is advised to use biosurfactants to increase the low water solubility of pesticides and herbicides, which will increase the rate of biodegradation.

When exposed to rhamnolipids at a concentration of 4 g/l, an acclimated culture of Alcaligenes eutrophus demonstrated the ability to mineralize 4,4-chlorobiphenyl up to three times more fast. Trifluralin and coumaphos biodegradation by microbial consortiums isolated from contaminated cow dip was enhanced by rhamnolipids and Triton X-0 in both liquid cultures and soil slurries. Biosurfactants are superior than synthetic surfactants at saturating herbicides, according to studies by Wattanaphon et al. The scientists examined the effectiveness of a biosurfactant of the glucolipid type from an isolated strain of Burkholderia cenocepacia BSP3 to synthetic surfactants in terms of increasing pesticide solubility. Methyl parathion, ethyl parathion, and trifluralin, three pesticides with varying water solubilities, were shown to be dependent on the surfactant and its concentration for efficient solubilization.

The biosurfactant from B. cenocepacia BSP3 considerably increased the apparent solubility of the three pesticides when the concentration was raised to 2 CMC, but an increase in the biosurfactant concentration within the range of 2-CMC had no appreciable impact on pesticide solubility. In the studies, the B. cenocepacia BSP3 biosurfactant performed better at increasing pesticide solubilization than Tween and SDS. Singh et al. identified a Pseudomonas species that was capable of generating bio-surfactant and digesting chlorpyrifos from agricultural soil by using an enrichment culture technique in the presence of chlorpyrifos. The ability of different concentrations of rhamnolipid in M-9 medium, ranging from 0. to 0. g/l, to improve chlorpyrifos partitioning to the aqueous phase was evaluated. Chlorpyrifos solubility in the aqueous phase rose from 2.5% in controls to >% in medium supplemented with 0.g/l of a biosurfactant formulation. The fact that the quantity of chlorpyrifos decreased along with an increase in biosurfactant concentration from 0. to 0. g/l, demonstrating the large increase in chlorpyrifos partitioning in the aqueous phase that the absence of rhamnolipids induces.Only 7% of the chlorpyrifos was broken down in the absence of the biosurfactant, whereas over % was broken down in its presence[3].

The hexachlorocyclohexane isomer class of pesticides is considered to include highly hazardous, carcinogenic, and endocrine disrupting chemicals. Due to their weak solubility in water and the spatial arrangement of chlorine atoms in the different HCH isomers, they are persistent in the environment. Rhamnolipids produced by an isolated strain of P. aeruginosa WH-2 improved the solubility of HCH isomers in water, suggesting a potential function for biosurfactants in the bioremediation of pesticides and herbicides.

Massive Metals

Direct exposure to industrial discharge, inadequate waste management, spills, and broken land disposal systems are all factors in soil contamination with metals. Metals including lead, chromium, cadmium, mercury, arsenic, copper, nickel, and zinc have been found in several sites. Contrary to many harmful organic constituents, metals cannot be readily detoxified or eliminated. The presence and fate of metals in soil are a subject of concern due to their possible impacts on microbial communities, danger of groundwater contamination, and toxicological effects on human health. Organic substances and colloidal ppapers are often closely related to metal contaminants in the environment. This is a considerable barrier to their removal using the current in situ cleanup techniques.

Although their method is distinct from that suggested for the removal of hydrocarbons, such as solubilization, mobilisation, sorption, and emulsion formation, biosurfactants may improve the removal of metals. Mulligan et al. looked at possible mechanisms for the removal of

metals from soil utilising surfactin. They postulated that the biosurfactant must directly interact with the sorbed metal contaminants to form complexes prior to detaching from the soil. The biosurfactant enables the removal of the metal from the soil's surface by reducing the interfacial tension between the soil colloid and metal. The metal and the biosurfactant micelle come together strongly, inhibiting the metal's read sorption into the soil [4].

DISCUSSION

The ions of Cd, Cu, lanthanum, Pb, and Zn are among the metals that are removed from soil by rhamnolipids, which are anionic and have the capacity to complex.The soil may be exposed to biosurfactants during ex situ soil cleansing methods. Because biosurfactants may foam, when air is introduced, metal-biosurfactant complexes may be removed by foaming. The biosurfactant can then be recycled by precipitation by bringing the pH of the solution to 2. Neilson et al. looked at the removal of Pb by rhamnolipids. A mM solution of rhamnolipid successfully eliminated % of the Pb from the soil after 10 washings. Lead removal was not significantly impacted by high Zn and Cu concentrations.

Rhamnolipids have been added to mining ores to enhance metal extraction. Batch tests were done at room temperature, and 2% rhamnolipid concentration was used to extract the Cu. The addition of 1% NaOH enhanced removal for a 2% rhamnolipid concentration by up to %, while removal decreased at higher surfactant concentrations. This study showed how important pH is to bio-surfactants' ability to remove metals. Sequential extraction experiments were conducted to characterise the mining ore and determine the kind of metals that the biosurfactants were removing. The oxide fraction, carbonate fraction, organic matter fraction (5%), and residual fraction (%) all included approximative amounts of the Cu. After six days of washing with 2% biosurfactant at pH 6, the carbonate fraction and the oxide fraction were gone[5].

The effect of pH on the capacity of rhamnolipids to remove metal was studied by Asci et al. Rhamnolipids produce vesicles that mimic liposomes and their rhamnosyl moiety is at least % unchanged at low pH. Between pH 6.0 and 6.6, rhamnolipids either form lipid aggre- gates or lamella-like structures. When the rhamnosyl moiety is negatively charged above pH 6.8, micelles form. This demonstrates that for rhamnolipids to act more efficiently, the pH should be higher than 6.8. The greatest Cd recovery efficiency was achieved using rhamnolipids with a starting pH of 6.8–7.0. Similar to this, rhamnolipids could only remove.8% of Zn from Na-feldspar at pH 6.8. On the other hand, the sophorolipids type of biosurfactant is better suited to low pHs as the surfactant solution transforms into a milky white solution with precipitate at pHs above 7.0. Following one washing with sophorolipid-containing solutions, the rates of Zn removal were 4, and 7%, respectively.

Kim and Vipulanandan investigated the removal of Pb from water and polluted soil using a biosurfactant derived from vegetable oil. The biosurfactant:Pb ratio for effective removal in the system was found to be 0:1, and at 10 times the CMC, more than% of the Pb was removed from water that had 0 mg/l of Pb contamination. To replicate a flow-through remediation method, Dahrazma and Mulligan assessed the effectiveness of rhamnolipids in a continuous flow configuration for the removal of Cu, Zn, and Ni from sediments collected from Lachine Canal, Canada. The elimination process was carried out by the biosurfactant's carboxyl group, according to FTIR spectroscopy. A rhamnolipid solution was continually sent down a column of sediment samples in this configuration. Without any other components, rhamnolipid alone was used to extract % of Cu, % of Zn, and % of Ni from the sediments. Cu elimination increased by up to four times with the addition of 1% NaOH compared to 0.5% rhamnolipid alone.

Juwaker et al. isolated the di-rhamnolipid biosurfactant from P. aeruginosa strain BS2. The effectiveness of isolated biosurfactants as a washing agent to remove metal from multimetal-polluted soil was also assessed. In the leaching trial, di-rhamnolipid increased the soil's ability to remove Cr by a factor of two compared to tap water alone, while Pb and Cu were removed by a factor of nine and two, respectively. Rhambolipids were particularly good in removing metal from polluted soil in a matter of hours. As opposed to tap water, rhamnolipids doubled the rate of Cd and Ni leaching from the soil that had been spiked. These results showed that the leaching behaviour of the biosurfactant differed depending on the metal.

In situ biosurfactant production and bioremediation of contaminated sites using immobilised microorganisms are thought to replace many present chemical and physical techniques. For instance, the use of immobilised cells may prevent freshly imported microorganisms from coming into direct contact with the local microbial population and can counteract detrimental environmental elements that impair microbial viability. Additionally, immobilisation makes it possible for recurring cell usage and makes it simpler to monitor the metabolism of bacteria. In order to biodegrade n-hexadecane and ultimately retrieve biosurfactant, Barreto et al. recently confined B. subtilis LAMI8 spores in chitosan beads that were afterwards cross-linked with glutaraldehyde. The cells in captivity destroyed almost 0% of the n-hexadecane in a medium with an addition of 1% glucose in a matter of hours. The number of living cells inside the beads was maintained throughout the experiment, and the biosurfactant that was emitted did not run out of carbon.

It has been proposed that the stability, storage, and long-term survival problems associated with vegetative cells may be resolved by entrapping bacterial spores in chitosan beads. This technique may be used to biodegrade complex compounds by encasing the spores of several bacteria types that generate biosurfactants. Mahanty et al. presented a novel technique for the biodegradation of PAHs by encapsulating pyrene in alginate beads for controlled release of pyrene and subsequent biodegradation by Mycobacterium frederiksbergense. Despite the fact that this strain does not produce biosurfactants, it is nevertheless feasible to measure the biodegradation of contaminants that have been immobilised using bacteria that do.Novel Strains for the Production of Bio surfactants. The primary need for bioremediation is the ability of microorganisms to survive and/or grow in contaminated conditions while efficiently degrading the contaminant. Even though a number of microorganisms have been noted for their effectiveness in bioremediation, there is still a need for efficient biosurfactant-producing bacteria, particularly for the development of in situ bioremediation processes[6].

The Uses of Biosurfactants in Agriculture

In order to hydrophilize heavy soils and achieve uniform fertiliser distribution, microbial surfactants are used in agriculture. Additionally, they make it easier for pesticides to be applied, disseminate, and penetrate the ground. They also prevent certain fertilisers from caking while being kept. A more complete examination is required to evaluate the potential of biosurfactants in agriculture since there are so few publications on the topic. Polluted water and soil may be cleaned up using biosurfactants. Biosurfactants are rapidly biodegradable and less dangerous than synthetic surfactants, according to research, yet the bulk of studies are confined to rhamnolipid ex situ treatment. It is vital to research the potential of substitute biosurfactants, such as sophorolipids and other bio-surfactants that may display superior attributes to those of rhamnolipids, in order to successfully develop suitable biosurfactant-based in situ treatment procedures[7], [8].

One method for removing metal-contaminated soils more quickly is phytoextraction with help from bioaugmentation. Due to the increased bioaccessibility of metals in soils, bio-augmentation often increases the amount of metal that plant shoots may accumulate by a ratio of two to five, with no obvious distinction between the effects of bacteria and fungi. Microbial siderophores, organic acids, and surfactants continuously control the bioavailability of metals. In contrast to bacteria, fungus immobilise metals in high-concentration environments. Sadly, the often low inoculant survival rate may lessen the impact of bioaugmentation. The formulations and management of microbial inoculants are covered in this chapter, along with techniques for selecting the ideal plant-microbe combinations for productive soil metal phytoextraction. Ecological engineering techniques might be used to maximise effectiveness in situations with variable conditions. The effects of the approach on the environment are investigated and the outcomes of field-scale studies are reported. The main topic of discussion when it comes to potential futures is how to maximise metal concentrations and amounts in plants.

CONCLUSION

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental pollutants that pose significant risks to human health and the environment. Microbial biodegradation is a promising method for the remediation of PAH-contaminated environments, and research in this area has led to significant progress in understanding the mechanisms and factors that influence PAH biodegradation.

The use of microbial technology for the biodegradation of PAHs has several advantages over traditional physical and chemical methods, such as cost-effectiveness, sustainability, and minimal environmental impact. However, the effectiveness of microbial biodegradation depends on a range of factors, including the type of microorganisms used, environmental conditions, and the presence of other pollutants. Research on PAH biodegradation has led to the identification of several microbial strains that are highly effective at degrading PAHs, as well as the development of novel bioreactor designs and bioremediation strategies. These advances hold great promise for the remediation of PAH-contaminated sites and the protection of human health and the environment.

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CHAPTER 18

A STUDY ON SOFT TECHNOLOGY IN MICROBIAL

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ABSTRACT:

Soft technology, also known as low-technology or appropriate technology, refers to the use of simple, low-cost, and sustainable techniques to address environmental and societal challenges. Soft technology has significant potential in the field of microbiology, where it can be used to develop innovative solutions for microbial detection, identification, and monitoring. This review provides an overview of the principles and applications of soft technology in microbial research, including the different types of soft technology, such as paper-based diagnostic tests, microbial fuel cells, and biosensors, and their advantages and limitations. We also discuss the factors influencing the performance of soft technology, such as sensitivity, specificity, and reliability, and the potential of combining soft technology with other techniques, such as microbial cultivation and molecular biology, to enhance the accuracy and precision of microbial analysis. Furthermore, we highlight the recent advances in soft technology, such as the use of nanomaterials and microfluidics, and their potential to revolutionize microbial analysis and monitoring.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

In contrast to organic pollutants, which may be broken down in the same soil matrix that has to be cleaned up, toxic metal remediation requires removal. Nonpoint Source Pollution, which contains intermediate metal concentrations throughout broad surfaces, has received little attention.When evaluating the situation in Europe and the USA, it is discovered that 0 hectares of land have been contaminated with heavy metals as a consequence of air deposits as well as regular use of pesticides and fertilisers that contain trace amounts of metals. Although the levels of metal are lower than in industrial regions, they are nevertheless high enough to contaminate the food supply and endanger the environment and humans. Crop production may go on after treatment thanks to a "soft technology" called in situ soil phytoextraction. When comparing alternative approaches to controlling contaminated agricultural soils, phytoextraction may have the potential to benefit farmers financially. This method's slow progress, which is brought on by metals' limited bioaccessibility, is often mentioned as one of its main shortcomings[1].

The availability of several synthetic chemicals to plants has been investigated, however there have been downsides such as low biodegradability, toxicity to plants, bacteria, and nematodes, risk of lixiviation, and high cost. Rhizoremediation is a practical choice that combines phytoextraction with soil bioaugmentation to enhance the beneficial effects of plants and microorganisms. Phytoextraction may be accelerated by the rhizobacteria's impact on plant growth, which increases plant biomass, or by the microbial inoculant's production of enzymes, siderophores, organic acids, or biosurfactants, which speed up metal absorption.

Furthermore, plants are crucial nutritional sources for supporting microbial growth and activity. The technical characteristics of metal bioaugmentation-assisted phytoextraction, i.e., the efficiency and control of the metal phytoextraction in settings with varying conditions, were defined by Lebeau et al. after a comprehensive analysis of the data that had previously been published. It has already been shown that plants often increase metal concentrations and total amounts absorbed by a factor of two and five, respectively. Since then, fresh research has verified previously reported results[2].

The basic mechanisms governing plant-microorganism interactions are reviewed in this chapter, followed by some helpful instructions on how to carry out on-site metal phytoextraction-assisted bioaugmentation from solid matrices like soil and silt. Ecological engineering may be a viable tactic for in situ metal phytoextraction-assisted bioaugmentation to obtain optimum efficiency and control in scenarios with changing conditions. Methods are also covered to provide a thorough understanding of the workings of this technique. Future scenarios are then presented.

Mechanisms for metal extraction in Plant-Microorganism Systems

The fundamental reason for the little quantities of metals that plants extract from soil and the slow rate of extraction is low metal accessibility. In actuality, metals that may be present in plants account for less than 1% of the overall metal composition. This raises the issue of how to differentiate between bioaccessibility and bioavailability. The definitions provided by Semple et al. state that a bioavailable compound is "that which is freely available to cross an organism's cellular membrane from the medium the organism resides in at a given time," whereas a bio accessible compound is that which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical.

Generally speaking, microbial processes that dissolve metals increase their bioaccessibility, while those that immobilise them decrease it. The balance between metal mobilisation and immobilisation represents the amount of metals that are available for the plant to absorb. The metal, the microbe, the environment, and physicochemical elements like pH, CEC, and organic matter content all affect how much is present. In combination with phytoextraction, solubilization offers a way for removing metals from soils via a bioaugmented process[3].

Heterotrophic Evaporation: Chemoorganotrophic bacteria acidify the environment by collecting CO2 via respiration or by effluxing H+ pumps as a consequence of the action of H+-ATPases. The ability of bacteria to produce metabolites also has the potential to cause leaching. The most efficient acids are citric acid, which is known to solubilize Ni and Zn, and oxalic acid for Pb in pyromorphite. Biosurfactants like rhamnolipids and surfactin produced by Pseudomonas aeruginosa and Bacillus subtilis, respectively, may also help in metal mobilisation. Biosurfactants facilitate metal desorption from the solid phase and mobilise metal that has been sorbed to the solid phase by producing micelles. The free metallic shape is additionally complicated by biosurfactants. Surfactin and rhamnolipids have been discovered to mobilise copper from the organic soil. In soil, siderophores of low molecular weight are produced at a rate of a few mol/L of soil solution. Siderophores may complex additional metals in solution, such as Cu, Ga, Mn, Ni, and Zn, although with a less strong attraction than they do for Fe. Injection of siderophore-producing bacteria in soil increased the mobility of lead and chromium, according to studies by Braud et al. Pots bioaugmented with Aspergillus niger and P. aeruginosa showed higher levels of Cr and Pb in the exchangeable section compared to nonbioaugmented soil, where neither metal was observed. According to Diels et al., the siderophore produced by Alcaligenes Eutrophus CH greatly increases the availability of Cd, Pb, and Zn via complexation and biotic leaching.

Acidophilic, chemolithotrophic bacteria that fix carbon dioxide get their energy from the oxidation of iron to iron and sulphur to sulphur dioxide.

Biomethylation: This reaction may be seen in the metals Ag, Hg, Pb, Se, Sn, and Te. In comparison to their comparable elemental forms, methylated metals are physiologically more transportable.

Redox Methods: Microorganisms reduce Fe and Mn oxides, resulting in strong metal absorption and improved metal mobility in the soil.

Control Mechanisms for Plant Metal Uptake

Depending on the metal's bioaccessibility, metallic state, and plant condition, roots either complex metals to organic matter or take them up as soluble forms. Within the rhizosphere, proton extrusion, phytosiderophores, or organic acids are traded for metal mobilisation. However, neither the pH of the rhizosphere nor the production of extra root exudates are related to hyperaccumulation. It has been shown that rhizospheric bacteria may encourage root exudation under certain conditions. Despite the fact that root exudates have a substantial impact on metal mobility, no study has shown the indirect impact of soil microorganisms on phytoextraction rate via promotion of root exudation.

After being absorbed by the root, metals in ionic form may become immobilised by interacting with carbonates, sulphates, and phosphates present in plant cells. But unlike phytochelatin and metallothionein complexation, which tends to immobilise metals in root vacuoles, complexation with other organic molecules such histidine, nicotianamine, and citrate seems to improve metal transport in the xylem. Indeed, it has been shown in a number of phytoextraction studies that adding citric and oxalic acids to the soil of the plant increased metal accumulation and the translocation factor.

Depending on the kind of metallic species, the rate of translocation varies; some move swiftly towards the leaves, while others accumulate in the roots. When the translocation factor exceeds 1, hyperaccumulator plants often sequester less metal in their vacuoles than nonhyperac- cumulator plants. Hyperaccumulator of Ni exposed to metal Histidine concentration in the xylem was raised by Alyssum species, Streptanthus polygaloides, and Berkheya coddii, which promoted metal transport and translocation. Other Ni hyperaccumulator species cannot, however, experience this impact[4].

The pace of phytoextraction is also influenced by the physiological condition of the plant and how it responds to stress. The plant produces metal chelators such phytochelatins or metallothioneins to chelate and detoxify the metals as part of its control of metal toxicity. Metal absorbed. It has been shown that the production of glutamylcysteine synthetase, a precursor to phytochelatins, boosts Cd accumulation and tolerance in Brassica juncea. By utilising bacteria to produce 1-aminocyclopropane-1-carboxylate deaminase, which breaks down ACC, an ethylene precursor that may impede root elongation in stressed plants, plants may become less stressed.

Real-World Issues and Tips for Bioaugmentation Assisted by Phytoextraction

It is important to keep in mind the definitions of bioaugmentation offered by El Fantroussi and Agathos, which is "the technique for improvement of the capacity of a contaminated matrix to remove pollution by the introduction of specific competent strains or consortia of microorganisms," and a more comprehensive definition proposed by Dejonghe et al., which is "this approach corresponds to increasing the metabolic capabilities of the microbiota," during the current discussion. In that sense, bioaugmentation is the same as increasing the gene pool

and, therefore, the genetic diversity of that area. Applications of soil bioaugmentation to plant nutrition include promoting plant development, reducing phytopathogens, enhancing soil structure, mineralizing organic pollutants, and bioaccumulating or biolixiviating inorganic pollutants. Rhizobium and Frankia have been used in bioaugmentation methods to increase plant growth, as have other non-symbiotic microorganisms like Azospirillum, Azotobacter, Burkholderia, Gluconacetobacter, Herbaspirillum, and Klebsiella, as well as Bacillus, Pseudomonas, Aspergillus, and Penicillium. In recent bioaugmentation techniques, rhizobacteria and arbuscular mycorrhizal fungi, which promote plant growth, have been employed to increase metal transfer to plants. Rhizoremediation is a revolutionary method that improves the biodegradation of organic pollutants by providing a habitat for bacteria that break down organic pollutants. Lebeau et al. have thoroughly examined the influence of symbiotic and nonsymbiotic microorganisms on phytoextraction efficiency.

Plant Symbiotic and Mutualistic Relationships

A mutualistic or symbiotic relationship between the host plant and the microorganisms used in improved phytoextraction may exist. Examples of symbiotic microorganisms include exo-symbionts, or those living outside the roots, meso- or endo-symbionts, such as AMF, which usually belong to the Glomales order and may colonise % of plant species. Depending on the plant-AMF relationship and the soil conditions, AMF may have a protective effect against metal toxicity and tend to improve plant tolerance while minimising metal deposition in shoots. Hovsepyan and Greipsson recommend employing a fungicide during the phytoextraction of Pb by maize in order to avoid metal immobilisation by AMF. Due to the enormous surface area of sorption and glomalin production, AMF may chelate metals in cells. According to many research, AMF bioaugmentation increased Cd accumulation in bean and maize, alfalfa, clover, and soybean. The findings vary depending on the kind of plant, for instance, Glomus intraradices inoculation increased Pb content in Agrostis capillaris roots but not in Zea mays. The efficiency of phytoextraction with AMF may depend on a number of variables, including the plant-microbial interaction, soil physicochemical properties, metal availability, inoculation conditions, and root density. Additionally, since the majority of hyperaccumulator plants are members of the Brassicaceae family, which is often not mycorrhizal and unrelated to AMF, the use of AMF in phytoextraction may be challenged.

DISCUSSION

Rhizoremediation might be more successful if it is bioaugmented with nonsymbiotic microorganisms since bacterial colonisation would be less reliant on the plant-microbe interaction. The most well-known microbial group, PGPR, might affect metal extraction indirectly by speeding up plant development as a result of P and K solubilization, indole acetic acid production (a phytohormone that promotes plant growth), ACC deaminase production (which breaks down ACC, an ethylene precursor that can obstruct root elongation in stressed plants), siderophores production, or directly increasing metal mobility with microbial metabolites are all examples of ways to do this.

P and K are often not readily accessible nutrients in polluted soils. The release of nutrients and the extraction of metals linked to P or K may both be increased by the inoculation of K- and P-solubilizing bacteria. Some microorganisms, like B. B was raised by subtilis SJ-1, which makes IAA. juncea metal tolerance and 1.5 times better Ni shot extraction. Experiments utilising naturally occurring ACC deaminase-producing bacteria, such as Kluyvera ascorbata SUD5, failed to detect any improvement in metal extraction, although plants transformed with bacterial ACC deaminase genes produced more metal than unaltered plants.

The development of chlorotic symptoms in plants growing on polluted locations is implied by the frequent connections between metal pollution, Fe deficiency, and plant stress. By acting as a source of iron for the plant, bacterial siderophores may boost the plant's growth, metal tolerance, and motility in the soil. Metal mobility in the soil is increased by siderophores, which are well-known as potent Fe chelatants and may also complex other divalent and trivalent ions. For instance, siderophores generated by Burkholderia cepacia were able to desorb Cd from goethite, while desferrioxamine B, a siderophore produced by Streptomyces, is able to mobilise Pb sorbed on goethite. A number of studies have found that metal phytoextraction and plant development are both enhanced by siderophore-producing bacteria.

Endophytic bacteria may be more suited than nonendophytic PGPR for aided phytoextraction because of their mutualistic interaction with the plant, which ensures greater root colonisation than would PGPR, according to Rajkumar et al. The processes for promoting plant growth and/or mobilising metals, however, are the same as for PGPR. Endophytic bacteria have been shown to facilitate Pb absorption and plant development by colonising the roots of Brassica napus and generating IAA, siderophores, and ACC deaminase.

Microbial Consortiums

To increase the rate of metal phytoextraction, several research have employed microbial consortia. The bioconcentration factor in B was raised by a bacterial consortia that had adapted to selenium. juncea by three when compared to the control, however the efficiency of phytoextraction dropped since the amount of biomass produced was lower. Coinoculation of AMF and B, according to a different research. cereus boosted Trifolium repens's ability to absorb Cd, Cr, Mn, and Ni. Consortia are often more effective than pure cultures since they may employ non-cultivable microbes and maintain their capacity to remediate.

Factors Undermining the Success of Bioaugmentation

The reduction in bacterial survival after inoculation owing to abiotic and biotic stress factors, as well as movement restrictions caused by microbial size and attachment, are limiting factors for the effectiveness of bioaugmentation. Due to their smaller size and higher mobility, the latter aspect favours the usage of bacteria rather than fungus. The encapsulation of cells within a matrix, the use of genetically altered microorganisms to transfer genes of interest in native microorganisms, the use of the rhizosphere as an ecological niche for introduced microorganisms, the use of ultramicrobacteria and bacteria that lack adhesives, and the use of surfactants to improve mobility are some of the techniques that have been developed to increase the efficacy of bioaugmentation[5].

The effectiveness of the injection technique and the selection of bacteria based on their rhizocompetence are the primary variables assuring soil colonisation success. A bacteria's rhizocompetence is measured by its capacity to utilise certain carbon sources and nutrients found in root exudates, mobilise iron, decrease nitrate, and carry out denitrification. Fast-growing bacteria are often present in young and immature soil organisms, and bacterial growth strategy is a significant component in soil colonization bacteria are slow-growing and tend to develop on established roots.

Due to their adaptability as a consequence of root maturation, phenotypical differences of microorganisms may also favour root colonisation, as shown by Pseudomonas brassicacearum's root colonisation of Arabidopsis thaliana. For bacteria to successfully colonise soil, siderophore synthesis and Fe mobilisation provide them a competitive edge over rivals or predators. The most competitive rhizospheric populations are members of the same siderotype, according to statistical analysis.

Genetically Modified Microbes

Instead of particularly enhancing metal mobility, the majority of modified microbes utilised in phytoremediation have been used to improve metal resistance, adsorption, and/or accumulation. The majority of research has been concentrated on modified microorganisms that can collect As, volatilize Hg and Se, and boost microorganism resistance to metals. a variety of B. Cepacia carrying the Ralstonia metallidurans Ni resistance system genes boosted root but not shoot Ni accumulation in Lupinus luteus.

Phytoextraction rates can be improved by genetically modifying microbes to produce more microbial metabolites. It has been shown that the Zn extraction by B is increased by an Enterobacter mutant, NBRI K SD1, that overproduces siderophores.

Plants

The majority of plants employed in phytoextraction should be able to effectively transfer metals from root to shoot, resist metals, grow quickly, and have a well-developed root system. .To increase the effectiveness of phytoextraction, a number of strategies have been devised, including the employment of hyperaccumulators, high-biomass plants helped by chemical or biological methods, fast-growing trees, and genetically modified plants.

Species with High Biomass vs. Hyperaccumulators

Remediation time, which might take years, is the main constraint for phytoextraction. A suitable and financially feasible period, i.e., less than five years, calls for the selection of species that are successful on weakly polluted areas and acclimated to their environment. The selection of high-biomass versus hyperaccumulator species is influenced by soil characteristics, metal speciation, and climate factors. Predictive models have shown that the nonhyperaccumulator Nicotiana tabacum is the most successful plant for Cd remediation, whereas the hyperaccumulator Thlaspi caerulescens is the most effective plant for Zn phytoextraction. When compared to employing high-biomass species for remediation of a broad region, the majority of hyperaccumulator species have low biomass and slow development rates. Z is an example. mays grown in the same field as T. Due to its increased biomass production, caerulescens can extract three times as much Cd, Cu, and Zn as the later species. Another research that tested the performance of several plant species on soil that contained 2.0 mg/kg Pb revealed that Z. Mays were twice as effective in extracting Pb as the best B. about three times greater than Thlaspi rotundifolium and juncea cultivar. As a result, several high-biomass plants, such as Avena sativa and B, have been employed in phytoextraction procedures. As well as B. pisum sativum, Z. napus, Helianthus annuus, Hordeum vulgare, and Pisum sativum. mays.

The production of hyperaccumulator species may be increased by fertilisation, as can their capacity for phytoextraction. Light NPK fertilisation is used B. With higher rates of N amendments, Ni content in shoots rose and coddii biomass increased double to tons/ha/year. Since excessive N fertilisation might lower the amount of Cd and Zn that a plant can absorb via T, fertiliser amendments must be tailored to each plant species. caerulescens and overly fertilised with P might even cause a decrease in biomass. Additionally, using P fertiliser might cause Pb to precipitate in the soil as pyromorphite and reduce the availability of metal.

The Function of Siderophores and Phytosiderophores in the Mobilisation of Metals by Plants

By secreting root exudates, protons, phytosiderophores, or organic acids, plants may mobilise or immobilise metals. PS are produced by strategy II plants, mostly graminaceous, in

mugenic or avenic acid forms or, in the event of Fe or Zn shortage, nicotianamines. Proton exudation, Fe reductases, and Fe transporters are all used by Strategy I plants to absorb Fe. However, PS are also capable of complexing several metals, including Cd, Ni, and Zn. Low-molecular-weight organic acids, which are also present in plant exudates, have the ability to mobilise Al, Ca, and Fe and boost the rate of their phytoextraction either by creating metallic complexes or by lowering the pH of the rhizosphere. Fe status of the plant and siderophore production in the rhizosphere have a close link. There are many plants, including H. pylori, Dianthus caryophyllus, and Lycopersicon esculentum. vulgar, Radiant Vigna, and Z. mays ,

A. hypogaea as well as A. Pseudomonas siderophores that are luminous may be used by thaliana as a source of iron. According to reports, plants preferentially utilise the bacterial siderophore ferrioxamine B over Fe-EDTA or Fe-PS, and it is quickly absorbed into Cucumis sativus's Fe-deficient Zones. The absorption and translocation of Fe by Z, however, is more effectively facilitated by mugenic acid than by FOB. For the absorption of Fe in wheat and barley, mays, PS, and a fungal siderophore called rhizoferrin are also more effective. These findings support approach I's use of C-style plants. sativus mostly collect siderophore-Fe complexes, while strategy II plants like Z. Mays have the capacity to synthesise PS and can take up PS-Fe complexes more easily.

The function of siderophore-producing bacteria in improved phytoextraction has not been extensively studied. Zn extraction was boosted by T by two times by Pseudomonas and Enterobacter. a caerulescens. P. aeruginosa, a siderophore producer, boosted the Pb and Cr phytoextraction from maize by 5.4 and 3.8, respectively, while R. raised the Cr shoot extraction by 5.2. metallidurans biological enhancement. According to another research, Streptomyces tendae secretes hydroxamate-type siderophores that boost H's absorption of Cd and Fe by %. Annuus fires.

Development of Plants

In addition to iron status, plant growth stage has a significant role in influencing the remediation process' duration and in lowering the cost of treating the biomass after phytoextraction. The concentration factor for Solanum nigrum's ability to remove Cd from the soil to the shoot was, in fact, lower at the mature stage than at the blossoming stage. Another research found that after two months of development, the As concentration in Pteris vittata fronds declined while it rose in the roots. Young plants with greater metabolic rates were shown to be more suited for phytoextraction[6].

Due to their deep root systems, flexibility to various substrates, and high biomass production, fast-growing trees are thought to provide suitable candidates for phytoextraction. The transfer of metal from root to shoot is aided in trees because of their high rate of transpiration; for instance, Salix may transpire,0 L of water in a single season. Salix may be modified for the treatment of agricultural soil polluted by Cd, according to an economic analysis.

Genetically Modified Plants

The majority of transgenic plants that are utilised in phytoextraction are used for selenium and mercury phytovolatilization. Several methods have been used to modify the plant's genetic makeup, including changing the selectivity of the absorption system and boosting the manufacturing of chelatant. An Arabidopsis plant that has been changed and can extract three times as much As as the unmodified plant has been created thanks to our understanding of the P. vittata As hyperaccumulation process. Metal tolerance and accumulation in the plant were both improved by increasing the synthesis of the chelant precursors glutathione and PC synthase. Moreover, A. received animal metallothionein genes. Brassica sp., Nicotiana sp.,

and A. thaliana. improved plant resistance to Cd but did not result in an increase in Cd accumulation, while the injection of P. sativum metallothionein into A. Cu absorption by roots was accelerated by thaliana.

Useful Tips for Choosing Plant-Microbe Pairs and Applying the Bioaugmentation-Phytoextraction Technique

Method for Selecting the Most Important Plant-Microbe Couples

It is necessary to choose the best plant-microbial inoculant combination in order to maximise metal extraction by plants. The microbial inoculant should have the following qualities: enough metal mobilisation from soil or sediment, sufficient survival rate, plant protection against metal toxicity, and encouragement of plant development. The primary criteria for plant selection are quantity of metals taken by a single plant species, root colonisation depth, biomass per soil surface area, metal accumulation and translocation, and Rhizodeposit availability and composition for microbial growth. The selecting process is made more difficult by the intricate interactions between these two partners.

The main issue with symbiotic microorganisms is that they are unable to alter the connections, and certain plants, such hyperaccumulators from the Brassicaceae family, which are normally nonmycorrhizal, do not participate in symbiosis. Contrarily, although AMF are nonhost-specific symbionts, host plants should get considerably more attention than they do given that the host plant plays the primary role in the symbiotic interaction. Endophytic bacteria have been proven to be able to withstand greater Ni concentrations than rhizospheric bacteria among non-symbiotic microorganisms. Unfortunately, the majority of endophytic bacteria cannot be grown. However, only biostimulation, not bioaugmentation, may be recommended. In contrast to Pseudomonas coming from the rhizosphere of another plant, Pseudomonas taken from the rhizosphere of maize and injected in the same rhizosphere demonstrated superior colonisation. Due to these restrictions, Kuiper et al. were the first to recommend choosing strains that are native to the rhizosphere of the host plant, dubbing this selection process "rhizo-directed strain selection."

Cocultivation of two or more kinds of plants is advised for two main reasons: first, certain plants, especially those in the Brassicaceae family, cannot be directly linked to AMF. On the other hand, T. In the presence of mycorrhized maize, caerule-scens and Sedum alfredii show increased Zn absorption. Associations of various plant species should be pursued and optimised with the goal of collecting the metal combination collectively. Unfortunately, the majority of research on multicontaminated soils focus only on the site's botanical makeup without examining the applicability of various plant associations for extracting various metals. Experiments should be conducted with the cropping of pertinent plant associations, where each plant is paired with the most suitable microbe[7].

Since bioaugmentation may reduce metal absorption by plants beyond specific concentrations, soil fertility and metal concentration are additional crucial factors to take into account when choosing plant-microorganism combinations. AMF-plant symbiosis is particularly applicable to less-polluted soils. with instance, with Cu concentrations less than 0 mg/kg of soil, maize that has developed symbiosis with the AMF Acaulospora mellea accumulates more Copper in shoots and roots than the nonmycorrhized plant. Andrade et al. discovered a comparable Cu concentration threshold for the Jack bean- Glomus etunicatum relationship. In the spectrum of metal concentrations examined, there was no change in the impact of bioaugmentation on metal extraction from positive to negative. For instance, using isolates of P. fluorescens G and Microbacterium sp., the effects of 0 mg Pb/kg and 0 mg/kg were examined. G; concentrations with soil infected with Bradyrhizobium sp. varied from 0

up to 0 mg/kg for Ni and 0 up to 9,0 mg/kg for Ni. .When plants are grown under ideal, stress-free circumstances, PGPR impacts on plant development may be diminished. When choosing a plant-microorganism combination, time and space constraints are also important considerations.

Even while microorganisms must eventually be evaluated on plants, a preselection might include growing microorganisms with synthetic root exudates since they are crucial for the establishment and development of bacteria in the rhizosphere. They would have distinct chemical and physiological makeups that mimicked various plant species. The make-up of root exudates originating from certain plants is already well understood, as is the variation in their make-up caused by factors like cultivars and photoperiod cycle. As a result, novel microbial culture medium with a structure resembling root exudates are recommended. More options might be examined since this preselection would significantly cut down on the amount of time needed for final selection. By using a single extraction technique and chemical extractants, the metal phytoavailability in soil may be ascertained using this selection scheme approach.

Microbial Inoculant Preculture Conditions

Lack of consideration for microorganisms' capacity to multiply in soil after bioaugmentation often compromises their ability to survive. In addition to the availability of nutrients in the soil, phenotypical traits of the microorganisms and inoculation processes prior to soil bioaugmentation, including inoculant preculture and packing, are connected to microbial persistence and activity. Bingemann et al. were the first to mention a "priming effect". Priming basically involves exposing an isolate or population of microorganisms to settings under which they are intended to operate in the future. The distinction between "priming" and "activated soil" is that "soil priming" tries to specifically choose the soil's microorganisms that will be cleaned up. Activated soil works by first "priming" a portion of the soil with the pollutant in order to choose the right microorganisms, and then it works by bioaugmenting the soil that has to be cleaned up. Without removing the degraders from the soil, activated soil functions as an inoculant, transporter, and supplier of nutrients all at once. The following are the key benefits of these techniques: availability of a variety of important supplementary microorganisms; availability of both cultivable and noncultivable microorganisms;

Since soil acts as a transporter for microorganisms, no processes of extraction and culture of microorganisms are required, which improves microbial lifespan. Increased microbial survival following soil bioaugmen- tation may also be achieved with the use of preculture media, such as soil extracts, whose compositions are similar to those of artificial culture media. According to theory, using microbial consortia rather than monoculture is more appropriate since they may collaborate to carry out more difficult jobs and hence surviving more unpredictable and challenging circumstances in polycontaminated settings. It is possible to employ both "natural" and "artificial" microbial consortia in bioaugmentation. Global performance may be optimal in the first scenario, but assemblages can maintain homeostasis because members typically do not outcompete one another or deplete the resources in their environments, and they can typically withstand harsh conditions as a result of long-term selection. Although the microbial communities that make up consortia are continually changing, the delicate balance between the many populations that make up the consortium is often maintained, giving these systems a high level of resilience[8].

Only the populations' proportions within the consortia may change. For instance, when nutrients are few, a minority group may become the most active. Thus, microbial communities are most likely the cornerstone of the management of microbial resources in the

field of environmental biotechnology. In contrast to natural consortia, manufactured consortia could be established by assembling already chosen microbes, such superbugs. But the long-term actions and outcome of such a coalition are unknown. Some bacterial groups, such as Glomus mosseae and Brevibacillus, G. Glomus spurcum, intraradices, and G. the mosseae and A. niger, Microbacterium saperdae, Pseudomonas mon- teilii, Enterobacter cancerogenes, a combination of six rhizobacteria, and Azotobacter chroococcum, Bacillus mega- terium, and Bacillus mucilaginosus were examined with the intention of improving metal phytoextraction. There were no discernible changes in the amount of metals that plants accumulated between pure cultures and microbial consortia.

Formulations and Management for Microbial Inoculants

Numerous research make use of bacteria that have been added during the liquid culture stage, which cannot ensure optimal bacterial dispersion, shelf life, or activity. Inoculum survival has been improved by the testing of inoculant compositions. Similar to how biofilms stabilise natural consortia, artificial immobilisation is a useful strategy for inocula stabilisation. By soaking seeds of Alyssum murale in bacterial solutions mixed with methylcellulose, the seeds were infected. Paxillus involutus was injected into willow cuttings from annual shoots using a peat-vermiculite substrate. There have been experiments with other peat compositions. Using immobilised P. aeruginosa cells in the soil that were fed with skim milk improved the absorption of Cr and Pb by maize shoots by a factor of two respectively, 5.4 and 3.8. a manufactured inoculum made of tested rhizobacterial strains.

As demonstrated by Braud et al., who compared skim milk supply with cell immobilisation in Ca-alginate matrix, it is often assumed that the main limiting factor affecting the cell survival and growth of inoculants is the amount of soil substrates available for microorganisms, rather than the ability of microorganisms to survive. However, cell immobilisation proved effective in shielding inoculants from grazing, interspecific rivalry, and environmental stressors such metal toxicity. Substrates that are precisely metabolised by the targeted microorganisms may help ensure their survival by being added to the soil.

Different inoculum sizes were investigated, but there is no evidence that they are connected to the rates at which plants extract metal. One might conclude that soil oligotrophy may hinder the development of biomass additions, as was stated above. Reinoculation attempts, with varying degrees of success, to upset the ecosystem's delicate equilibrium in favour of the inoculum. When using seed soaking for bioaugmentation, the microbial inoculant has to be closely bound to the seeds. As an alternative, soil may be bioaugmented with AMF at the time of planting or even earlier.

Planting Density and Culture Duration

Regarding the length of the culture, investigations may be categorised as short-term, often carried out in lab and greenhouse circumstances, and long-term, in field conditions. Rarely does the literature discuss whether or not the time of harvesting coincides with the target metal's maximum uptake by plants. Furthermore, kinetic investigations are very rare, yet plant-useful nutrients are required at varying times during the growth season. Jankong et al. demonstrated in a field experiment with the fern Pityrogramma calomelanos that the quantity of As in fronds was somewhat greater at weeks than at 6 weeks, but the amount in roots was lower. Higher As accumulation in roots and fronds was seen in a nonbioaugmented control after 6 weeks. Similar results were found by Chen et al. for the accumulation of As and 8U by another fern that had been mycorrhized with a different species of Glomus and collected after 8 weeks.

According to the stage of plant development, rhizospheric biomass is also significantly changed. When connected with B, juncea populations decline as follows: seedling stage > blooming stage > tillering stage. the juncea. Within a few days of the inoculant being added to the soil, plants have access to the extra metal. Following Burkholderia sp. soil inoculation, peak levels of water-soluble Pb and Cd in soil solution were attained and h, respectively.J . The readings corresponded to a 2 pH unit drop. Similar findings were reported by Braud et al. for agricultural soils bioaugmented with P. fluorescens, P. aeruginosa, or A. and polluted with Cr and Pb. niger. The uptake of components by plants from the environment is also influenced by planting density. The "target-neighbour" cocropping method may be used to analyse how planting density affects metal absorption. This approach is based on the ecological idea that competition for few resources grows with population density.

Experiments Done at a Field Scale

Only a small number of tests have been carried out in real-world settings. Both laboratory- and field-scale investigations were carried out by Belimov et al. Both pot studies and field experiments demonstrated the beneficial effects of rhizobacteria on the absorption of Cd and Pb by barley. In greenhouse and field tests for As phytoextraction, Jankong et al. discovered similar results using the fern Pityrogramma calomelanos bioaugmented with rhizobacteria. The validation of laboratory data at the field scale needs to pick up in the next years.

Financial Aspects of the Method

It has long been known that using plant aerial parts as a raw material for the creation of renewable energy may make phytoremediation cost-effective. On the other hand, when bioaugmentation is connected to phytoextraction, the additional benefit to the cost ratio is uncertain.Ways to Understand the Mechanisms Associated with Bioaugmentation-Phytoextraction Processes Better.

Techniques for Monitoring Inoculant, Microbial Diversity, and Microbial Activity

Traditional microbiological methods often undercount the quantity of microorganisms present and are unable to discover noncultivable bacteria. Molecule-level methods been created to keep track of injected microbes, including changes within microbial ecosystems. Information on the species composition of communities may be obtained using a variety of methods. Since various DNA extraction methods provide varying results during fingerprinting, normalised extraction techniques should be employed. Although real-time PCR allows for the identification of fewer than 4 cells/g of soil, DNA hybridization methods have been used to identify in situ microorganisms in environmental samples. However, they may not be as sensitive as PCR approaches to monitor tiny populations. S rRNA-based approaches are more effective than DNA-based ones because they allow for the monitoring of metabolically active cells and gene expression. Techniques for genetic fingerprinting describe population presence and variation. Numerous effective methods have been used, some of which may be combined with sequencing, such as T-RFLP, DGGE, TGGE, ARDRA, SSCP, DHPLC, and ARISA. The two most popular methods among these are DGGE and T-RFLP. When these two methods are compared, it seems that T-RFLP is more sensitive than DGGE.

By Gentry et al., methods to track microbial survival have been evaluated. Reporter genes, as opposed to fingerprinting methods, are used to monitor injected bacteria and enable differentiation from genetically similar infectious bacteria. These quantitative techniques mainly use the lux and gfp genes. As shown by Unge et al., who tracked P. fluorescens SBW survival in soil following tagging with a gfp-luxAB cassette, dual tagging approaches may be

used to detect both the presence and activity of injected bacteria. In situ PCR is less sensitive than quantitative PCR for counting particular injected bacteria, but both techniques may be used to monitor the specific gene expression of infected microorganisms. Detecting microorganisms at various levels, such as species or groups of species, and providing an indicator of microbial presence and/or metabolic activity in situ are both possible with the help of fluorescence in situ hybridization. Then, using epifluorescence microscopy or flow cytometry, cells may be counted. Recently, DNA microarrays were created to measure the amount of bacterial DNA and to assess the level of gene expression in microbial communities. The fundamental benefit of microarray technology is the ability to concurrently monitor the expression of many different genes. For instance, microarrays were used to track the expression of Ralstonia eutropha JMP4 genes in mixed microbial communities as a function of 2,4-D addition. Proteomics investigations work in conjunction with genomics to categorise microbial responses to environmental factors and to identify important enzymes and compounds in bioaugmentation and bioremediation processes. Moreover, by identifying proteins involved in their interaction, such as signal molecules, communication between plants and bioaugmented microbes may be evaluated. Proteomic investigations, for instance, have permitted the investigation of the PGPR impact of on the rice, P. fluorescens.

Methods to Calculate Metal Bioavailability Using Physical, Chemical, and Biological Data

According to Semple et al., up until now, the majority of common chemical approaches have generally estimated the bioaccessible fraction of metals, which must be taken into account as a matter of priority in the context of bioremediation. Different analytical approaches have been proposed, and estimating metal bioaccessibility relies more on the metal's form in the soil than it does on its overall quantity. These include several bioassays and physical/chemical extraction methods. On the chemical speciation of metals, much environmental science study has been conducted. It is becoming more clear that chemical elements' mobility, distribution, and biological availability rely not only on their concentrations but also on the physical and chemical interactions they experience in natural systems.

Two different approaches are usually applied in speciation studies for solid samples: single and sequential extraction procedures with reagents having different chemical properties. The extractants of choice imitate the impact of various environmental factors, including acid rain, on the fictitious release of metals from soil. Compared to sequential extractions, single-step extractions are quicker and less costly, but they don't reveal any connections between various soil fractions and metal concentrations. The most popular extractants for straightforward extraction in a single step are distilled water, DTPA, NaNO3, or CaCl2. A technique created by Feng et al. mimics the effect of root exudates on metal mobility by using a combination of acetic, lactic, citric, malic, and formic acids.

For sequential extraction, numerous schemes have been put forth. In a review, Filgueiras et al. listed more than 0 publications from the previous ten years that dealt with sequential extraction of metals. Some of these schemes might be slight variations of one another with different extractants or operational settings. Because redistribution and adsorption typically occur during extraction, the results obtained with these procedures are, of course, operationally defined. It is possible to see evidence of anoxic sediments being oxidised by the extraction reagents. The pH, extractant concentration, extraction time, temperature, and physicochemical characteristics of the soil all affect how effectively materials are extracted. Metal speciation is greatly influenced by the ppaper size distribution, drying temperature, and consequently presample processing.

Chemical extractants frequently overestimate the levels of phytoavailable metals, and the relationship with shoot metal extraction is not always accurate. It has also been demonstrated that the effect is highly dependent on the type of plant and metal used. However, the knowledge gleaned from these chemical methods is extremely valuable for evaluating metal reactivity, and consequently availability to the environment, and their potential negative effects.

Studies have shown that the amount of free ionic metal in the soil solution is an accurate predictor of the amount of biodegradable material that plants extract. Diffusive gradients in thin films is a novel method that has recently been developed to evaluate the phytoavailability of heavy metals. This method revealed a low correlation with Cu uptake by Z but a strong positive correlation between DGT-extracted Cu and Cu concentration in Lepidium heterophyllum shoots. mays . Similarly, a poor correlation was demonstrated between DGT-measured U and U concentration in ryegrass shoots .

The soil solution recovered by centrifugation is an alternate means to estimate this availability , requiring, however, a per- centage of soil moisture above %. If chemical methods are not always relevant to measure bioavailable metal concentration, biological tools such as biosensors can be used. Significant correlations have been reported for Ni concentrations detected by the biosensor BIOMET, derived from R. eutropha CH, and Ni accumulation by maize . Other biosensors have been created to assess bioavail- able concentrations of Cr and Pb in soil with R. metallidurans AE and Hg with a mer-lux Escherichia coli . However, none of these biosensors has been used to predict metal uptake by plants.

Efficiency of Phytoextraction-Assisted Bioaugmentation

Evaluation of Phytoextraction Efficiency Must Incorporate Several Parameters

Plant Parameters

A minimum value of the metal concentration in aerial portions must be attained. The cost of biomass treatment decreases as metal concentration in the plant increases. Nonetheless, the key parameter for efficient phytoextraction is the amount of metal extracted by the aerial parts of plants and per surface area, taking into consideration both metal concentration and plant biomass.

Accordingly, hyper- accumulating T. caerulescens whose Cd concentration in shoots was ten times higher than that recorded in Salix spp. extracted one-half Cd per hectare because of low biomass production . Because of their high biomass and extensive root system, trees are thus considered to be attractive for phytoextrac- tion, although metal accumulation tends to be low. Other parameters to evaluate phytoextraction efficiency include translocation and bioconcentra- tion factors , settling depth, ease of “mechanical harvesting, and the period and number of harvests expected per year based on phytoextraction rate .

Microbial Parameters

Microbial survival is an important parameter, but microbial activity appears more appropriate, since rate of metal extracted by plants and plant growth are not always shown to be correlated with inoculum size . Root elongation, solubilization of insoluble phosphates, activity of enzymes such as ACC deaminase, and IAA production are also relevant parameters that contribute to estimation of bioaugmentation relevancy.

Efficiency of Phytoextraction-Assisted Bioaugmentation

In spite of the numerous experimental protocols and various means of exploiting results, some tendencies can be derived from the extensive data published on this topic . What emerges is the following: various metals and metalloids have been studied multicontaminated soils and soils contaminated for long periods each represented only % of the experiments, and most have been conducted with soils from industrial origin; about % of soils were steril- ized exacerbating the positive effect of bioaugmentation; most experiments were performed in carefully controlled conditions and only a few in field conditions; and microorganisms were selected from various environments such as the rhizosphere where microorganisms seem more tolerant to metals than in the bulk soil. Microbes were selected for their tolerance to metals more than for their ability to compete indigenous microorganisms and protozoa and in a few cases for their beneficial interaction with plants and for their effect on the bioavailability of metals for plants.

The effect of microorganisms on plant biomass production and concentration of accumulated metal is shown in Table .2. The amount of metals extracted by shoots resulted in two main situations: an increase of plant biomass simultaneously to a decrease of metal concentration accumulated in plants. In such cases, bioaugmentation enhanced metal phytoextraction by a factor not exceeding 1.5 up to 2; and the increase of both plant biomass and metal concentration as observed in a few studies and resulting in a fourfold higher metal amount in shoots with a maximum value that reached a factor of . Most often, PGPRs

As with any in situ remediation process, the environmental impact of bioaugmentation-assisted phytoextraction must be evaluated. Indeed, one may be concerned that the increase of metal availability is greater than the plant accumulation ability with a risk of contamination of subsoil and groundwater. This phenomenon has been observed for EDTA-assisted Pb extraction, resulting in more rapid mobilization of Pb by EDTA than its uptake rate by Brassica rapa L., Vigna radiata L., and Triticum aestivum L. .Nonetheless, the same authors have shown that Vetiver zizanioides is able to recover all Cd, Cu, and Pb complexed with EDTA as a result of a well- developed root system.

Some bioindicators of the recovery of soil functioning following phytoextraction or bioaugmentation have already been used. Di Gregorio et al. have observed a severe modification of the bacterial community structure of the soil, using Denaturing Gradient Gel Electrophoresis , due to cultivation with B. juncea. Conversely, EDTA only slightly affects bacterial community structure, with the exception of the simultaneous presence of B. juncea and PGPR Sinorhizobium sp. In contrast, pseudomonads inoculated in soils, either uncontaminated or contami- nated with Cd, induced a shift in microbial communities, as suggested by analyzing in situ catabolic potential . Conversely, Epelde et al. showed a soil functioning restoration following Cd and Zn phytoextraction as reflected by the values of different microbial parameters. Metal phytoextraction level also affects microbial activity and community composition.

During the past years, microorganism-assisted phytoextraction has generated numerous experiments that have clearly shown the potential for increasing the quantity of metals extracted by plants. Although we now have a much better understanding of how microorganisms and plants interact in enhancing metal phytoextraction, one can wonder how great the concentration and amount of metals in plants can become. Some questions are still in abeyance, thus limiting the success of such a technique. For example, is the pres- ence of inoculated microorganisms the limiting factor affecting metal bioaccessibility or the maximal amount of metals accumulated by plants? Low bioaccessibility of metals in soil is often

pointed out as the major limitation in phytoextraction efficiency. Soil bioaugmentation by siderophore-, biosurfac- tant-, or organic acid-producing microorganisms were shown to significantly enhance metal bioavailability , siderophores being the most efficient.

Nonetheless, Glick stated, in his review, that PGPR producing such molecules would only slightly modify metal accessibility in the presence of high concentrations of metals. To definitively conclude with that point, mass bal- ances should be undertaken to estimate the surplus bioaccessible metal in the soil following bioaugmentation and to conclude whether the entirety is extracted by the plant or not. Braud et al. showed that, in spite of a higher amount of Cr and Pb in both the bioaccessible fraction of the soil and in maize shoots following soil bioaugmentation, the amount of metals accumulated in the whole plant almost always decreased. This imbalance between metal supply and uptake was already observed when synthetic metal chelators were used . Consequently, the concern persists that some metals will leave the root zone and generate an environmental risk that must be evaluated.

Regarding microbial–plant interactions, Audet and Charest underscored a transitional role of AMF symbiosis in phytoremediation, shifting from “enhanced uptake” to “metal-binding” beyond critical metal levels. PGPRs seem less suscep- tible to metal load in the soil , and bioaugmentation could be performed at higher metal concentrations. Although some microbial activities were identified, i.e., IAA, siderophores, and ACC deaminase, in promoting plant growth, further research should unravel the contribution of each. In his review, Glick reported that the presence of all or even some of these activities could be sufficient to elaborate the entire mechanism regarding plant growth promotion. Using mutants not producing or overproducing these microbial activities will be very useful to better understand the mechanisms involved in plant growth and metal phytoextrac- tion following bioaugmentation. An increased tolerance of Medicago truncatula was shown with IAA-overproducing strain Sinorhizobium meliloti DR- as the result of proline accumulation and decreasing levels of the antioxidant enzyme superoxide dismutase, peroxidase, glutathione reductase, and ascorbate peroxidase. Some rhizosphere chemical dialogues reviewed by Badri et al. also occurred in the intricate microorganism–plant interactions. For example, a specific plant signal could be received by each rhizobacterium, making the microorganism-plant selection much more complex. Single host–single PGPR interactions are most often examined while multiple interactions exist in nature. The same authors stated that although abundant information is available on the role of root-secreted secondary metabolites in rhizospheric plant–microbe interactions, the role of exuded proteins is poorly studied.

There is also a need to clarify whether soil bioaugmentation effects on plant growth and metal phytoextraction are direct or indirect via indigenous microorgan- isms whose structure and activity could be altered by the supply of exogenous microorganisms. Kinetic studies should also be performed to analyze both the length of time before plant harvest and the balance in the course of the time between surplus bioaccessible metal following bioaugmentation, and metal uptake by plant. Indeed, it is never mentioned whether or not time of harvest corresponds to the maximal accumulation of metal in plants, while it is well known that nutrient needs vary greatly as does biomass during the growing period. For example, Cd concentra- tions in roots and leaves of the hydroponically cultivated Allium schoenoprasum increased until 7 days and were stable afterward until days at the two concen- trations of Cd tested while the biomass increased. With B. rapa , Ag, Al, and Si concentrations decreased over the course of the growing cycle, while B and Ca increased with crop age . Regarding surplus bioaccessible metals following bioaugmentation, peak values of water-soluble Pb and Cd in soil solution were attained h and h, respectively, after

soil inoculation with Burkholderia sp. J . Similar results were attained after 1 week, at most, with P. aeruginosa, P. fluore- scens, and A. niger for Cr and Pb . The physiological state of the plant associated with the inoculant also plays an important role. With B. juncea, PGPR population size varies according to the growth stage of the plant: seedling stage > flowering stage > tillering stage.

CONCLUSION

The primary objective of process engineering, in situ bioaugmentation-assisted phytoextraction, will likely come up in the near future. However, given the spatial and temporal variability of open ecosystems, how well will we be able to control this process? According to Vogel and Walter , microbial ecology issues are among the most important in bioaugmentation approaches, although, unfortu- nately, they are rarely addressed. The ecological engineering concept first defined in the s should be taken into account in all experiments regarding microorganism-assisted metal phytoextraction. This suggests using engineering technologies based on ecological principles to design, develop, and maintain ecosystems. According to Verstraete et al., microbial communities are most likely the cornerstone of the management of microbial resources in the field of environmental biotechnology. The difficulty for microbial ecologists is to expand the soil metabolome by introducing pertinent microorganisms so that the system can benefit from their actions.

To conclude, future prospects for in situ bioaugmentation technologies should be to reconcile process engineering, based on the full control of any system, with variable environmental conditions. Systems associating microorganisms and plants are relevant, since physicochemical conditions in the rhizosphere are less suscep- tible to change in the course of the remediation effort than in the bulk soil. Additionally, compared to the soil oligotrophy, rhizospheric soil contains a higher amount of nutrients regularly exuded by the plant , ensuring a continuous substrate supply to microorganisms. Therefore, rhizosphere bioaugmentation could be thought of as a way to improve microbial survival, root colonisation, and metal supplies to plants.

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CHAPTER 19

FACTORS AFFECTING URANIUM(U) ACCUMULATION BY BACTERIA

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ABSTRACT:

Bacteria play a vital role in the biogeochemical cycling of uranium (U) in the environment, including its immobilization and solubilization. The ability of bacteria to accumulate U is influenced by various environmental factors, such as pH, temperature, nutrient availability, and the presence of other metals and organic compounds. This review provides an overview of the factors affecting U accumulation by bacteria, including the mechanisms involved in U uptake and accumulation, such as adsorption, complexation, and reduction, and the factors that influence these processes, such as bacterial cell surface characteristics, U speciation, and the presence of other competing ions. We also discuss the applications of bacterial U accumulation, such as bioremediation of U-contaminated soils and waters, and the challenges and limitations associated with this process, including the potential for bacterial toxicity and the need for the development of more effective and sustainable bioremediation strategies.

KEYWORDS:

Bacteria, Microbial, Microorganism, Microbial Cells.

INTRODUCTION

For a better understanding and optimisation of microorganism assisted phytoremediation, experiments based on multifactorial designs are required to elucidate both the respective contributions of the key factors involved in metal phytoextraction and their interactions. Unfortunately, only a few parameters are evaluated at a time, resulting to a lack of certain default values, therefore precluding a thorough knowledge of ecosystem functioning. The Taguchi method of experimental design is a straightforward statistical tool that enables the estimation of the greatest number of main effects with the fewest possible experimental runs. By taking into account a large number of variables over a particular region of interest, proper experiment design aids in learning about optimised levels. This strategy focuses on how variation affects process characteristics. The suggested approach makes it easier to understand the intricate bioremediation process mathematically and to optimise nearly ideal design parameters. For example, among eight biotic and abiotic factors, substrate loading rate showed significant influence on bioremediation process.

Contrary to laboratory settings, open ecosystems expose microorganisms to a variety of continually changing environmental pressures, making it difficult to apply the knowledge gathered at one site to the development of treatment plans for other systems and pollutant kinds. Therefore, it is necessary to build stress response systems as instruments for efficient and comprehensive process management in order to provide predictions about microbial adaptive resilience. Modeling is also a technique of anticipating bioremediation efficacy and

to prevent too many tests, but they are, nevertheless, unable to properly reflect the immense complexity of ecosystems. Future possibilities should examine the function of plants in bioremediation as a microbial regulatory component[1].

Attempts were made to recover uranium occurring in nuclear fuel effluents and mine tailings using bacteria isolated from U deposits in Canada, the United States, Australia, and Japan. Hundreds of microorganism strains were screened in order to determine which microorganisms accumulate the most U. Some bacteria isolated from North American U deposits had extremely high U accumulating abilities. Arthrobacter and Bacillus sp. accumulated approx. 2,0 □mol U/g dry wt. of microbial cells during 1 h. With remarkable efficiency, cells extracted U from wastewater used for refining. Additionally, thorium was accumulated by cells very effectively. More U was removed from seawater by Lactobacillus cells isolated from Japanese U deposits than by any other bacteria with higher U removal ability from nonsaline U solutions. Polyacrylamide gel immobilised cells exhibited good handling qualities and could be utilised repeatedly in U adsorption-desorption cycles. These bacteria from the U deposits can be used as an adsorbing agent to get rid of the radioactive elements that could be in waste from other sources, such as mine tailings, seawater, and nuclear effluents.

The recovery of nuclear fuel components from aqueous systems, such as uranium and thorium, has attracted attention for the exploitation of undeveloped energy sources. Another important priority for environmental protection initiatives is the elimination of radioactive substances and toxic heavy metals from contaminated sources. In this regard, research efforts have mainly focused on understanding how bacteria, fungi, and yeasts accumulate U. We used bacteria isolated from U mines to explore the accumulation of U in aqueous environments. Some of these strains were discovered to have very high U accumulating capacities. Thus, the use of microbial biomass as a removal agent for the recovery of U from metallurgical effluents, mine tailings, seawater, and other waste sources may be taken into consideration. In U deposits, it may be expected that certain microorganisms having a high accumulating capacity for U and various species having an ability to leach U from ore may occur in mine soil and aquatic systems. It would, therefore, be helpful to identify bacteria with an improved capacity to collect U from mines[2].

Recently, we tested hundreds of different types of microorganisms found in uranium deposits in North America, Australia, and Japan for their capacity to accumulate significant amounts of uranium. As a result, we discovered new strains that did so, including Bacillus subtilis in Australia, Arthrobacter and Bacillus sp. in North America, Lactobacillus and Bacillus sp. in Japan, and Bacillus subtilis in Australia. Arthrobacter, Lactobacter, and Bacillus sp. in particular are highlighted in this chapter for their ability to remove nuclear fuel components like U from saltwater and wastewater used in the refining of U. Other novel bacterial strains discovered in North American, Australian, and Japanese U deposits are also described. To determine the ability of microorganisms isolated from U deposits in Canada, the United States, Australia, and Japan to accumulate U, hundreds of strains of micro- organisms were screened.

The medium for culturing microorganisms contains 3 g/L beef extract, 5 g/L peptone, and 5 g/L NaCl in deionized water. The bacteria were cultivated in 0 mL of the medium in a 0 mL flask with continuous shaking for h at °C while being kept on agar slants. Cells were collected by centrifugation, washed thoroughly with deionized water, and then utilised in the following accumula- tion experiments Arthrobacter and Bacillus species from the US, Lactobacillus and Bacillus species from Japan, and Bacillus species from Australia were

found to have extremely high U accumulating abilities and to produce large amounts of U per gramme dry weight of microbial cells in just five minutes[3].

Factors Affecting U Accumulation by Bacteria

In order to obtain basic information regarding the removal of U using strains of bacteria found in U deposits, some factors affecting U accumulation were investi- gated in detail using Arthrobacter sp., US- isolated from United States U deposits.

Effect of pH on U Accumulation

0.1 M HNO3 or 0.1 M NaOH were used to get a test solution's pH to the appropriate level. For 1 hour at °C, dormant Arthrobacter cells were suspended in a 0 mL solution containing M U. Additionally, bacteria at pH 2-9 in a solution of 0.0 M NaNO3 were used to evaluate Zeta potential by electrolysis. Utilising Arthrobacter cells, the levels of absorbed U are lowest below pH 4 and increase with increasing acidity between pH 5-8. The amounts of absorbed U using Lactobacillus, on the other hand, were highest at pH 6, and they rapidly decreased below pH 5 and above pH 7. Higher proton concentrations, which can compete with U for binding, naturally result in low pH, and it is evident that Arthrobacter cells were less impacted by the lowering pH than Lactobacillus cells until about pH 4. The low proton concentration and increased hydroxide ions, which compete with microbial cells, are the cause of the high pH, on the other hand. Again, Arthrobacter displayed a larger pH range for binding capacity than Lactobacillus, attaining acceptable absorption at pH values as high as 8, whereas Lactobacillus performed well only at pH 6.

Additionally, across the whole measured range of U concentrations, U absorption utilising these organisms, particularly Lactobacillus sp., does not follow the Langmuir isotherm. It seems that the experimental results reflect a twofold pattern. The dotted line was calculated using Lactobacillus cells and a residual U concentration of less than.8-M. Separate calculations were made for the solid line using residual concentrations at and above.8 M. The absorbed amount grew to values that were much higher than those predicted based on the connection in the low concentration range when the starting U concentration climbed beyond.0 M.

On the other hand, a residual U concentration below 3 M was used to calculate the dotted line in Fig. 3 for Arthrobacter cells. The solid line was independently determined for Arthrobacter cells using residual values below and above 3 M. The absorbed amount grew to values that were somewhat higher than those predicted based on the relationship in the low concentration range when the starting U concentration went above 8 M. Barley roots were used to absorb potassium, and the results were identical. A high Qmax value of 2,0 and 2,0 mol U/g dry wt. cells is determined from the high U concentration area employing Arthrobacter and Lactobacillus cells, respectively[4].

Resting cells were suspended in 0 mL solution containing M uranium for 1 h at °C. Three times using mL of mM EDTA solution were used to wash cells that had accumulated U.About,, and% of the accumulated U was desorbed from the resting cells when cells of Arthrobacter sp., US-, Bacillus sp., US-9, and Lactobacillus sp., JPN- were washed with EDTA. This finding suggests that most U is coupled with ligands that are easily substituted by EDTA. However, EDTA washing that was integrated inside cell membranes did not replace,, and% of the accumulated U in Arthrobacter, Bacillus, and Lactobacillus sp. The ratio of released U happened in the following order: Arthrobacter sp. > Lactobacillus sp. > Bacillus sp.

Distribution of U in Microbial Cells

The goal of the current experiments was to identify which cell components in Arthrobacter US- and Lactobacillus JPN- cells had accumulated U.Arthrobacter and Lactobacillus resting cells were suspended for 1 hour at °C in a 1.0 mL solution containing 0 M of U. The cells were divided into groups as shown in Fig.Arthrobacter that has been dried and frozen[5]

Immobilised Bacteria's Ability to Recover U

Bacteria including Arthrobacter, Bacillus, and Lactobacillus sp. may acquire significant amounts of U from aqueous environments, as was previously mentioned. However, due to their mechanical instability and susceptibility to cell degradation, these bacteria's free cells cannot be used again. Free cells should not be used in column systems because they can cause plugging. Arthrobacter sp., US- cells were immobilised with polyacrylamide in order to compensate for these inadequacies with free cells. An isotonic NaCl solution containing 4.5 mL was used to suspend five grammes of precultured Arthrobacter cells, along with 0 mg of acrylamide monomer. The suspension received a total of mg N, N-methylene-bis, 0.3 mL 3-dimethylaminopropionitrile solution, and 0.1 mL potassium persulfate solution. The gel was broken up into small pieces after solidification, thoroughly washed with isotonic NaCl solution, and then used for experiments on adsorption.

After six iterations of the adsorption-desorption cycle, the immobilised Arthrobacter cells' capacity to adsorb U did not diminish. Therefore, it appears that immobilised microbial cells have excellent handling properties and can be used repeatedly in adsorption-desorption cycles created at pH levels higher than 7. Arthrobacter and Bacillus cells, which can remove a lot of U from nonsaline water, removed much less U from either seawater or decarbonated seawater than did Lactobacillus sp., which only removed 2% of U from seawater but nearly twice as much when the seawater was decarbonated. Therefore, using Lactobacillus to remove significant amounts of U from seawater has a lot of potential[6].

We isolated bacterial strains with a considerable capacity to collect U from U deposits found in Canada, the United States, Australia, and Japan. These strains include Arthrobacter, Bacillus, and Lactobacillus sp. Within an hour, these species could amass approximately 2,0 mol U/g dry weight of microbial cells. These strains accumulated U selectively from solution containing six other heavy metals in solution. Also efficiently accumulating Th and U were cells. These species efficiently extracted U from effluent from the uranium refining process. Additionally, Lactobacillus accumulated U from a nonsaline U solution from seawater more efficiently than other microbial cells with strong accumulating capacity. Cells immobilized with polyacrylamide gel have excellent handling characteris- tics and can be used repeatedly in U adsorption desorption cycles. These strains of Arthrobacter, Bacillus, and Lactobacillus may be employed as an adsorbing agent for the removal of nuclear fuel components which may be found in nuclear fuel processing effluents, mining tailings, saltwater, and other environmental sources.

DISCUSSION

Waters contaminated with pollutants such as metals and dyes that are difficult to biodegrade can be cleaned up using bacterial biosorption. Numerous biomaterials, including as bacteria, fungus, algae, and certain industrial and agricultural wastes, are known to bind these contaminants. Alternatives that are less expensive and more efficient for removing metallic elements, notably heavy metals, from aqueous solutions include biosorbents. The sorption capacities of bacterial biomass towards metal ions are highlighted in this chapter. The ideal circumstances for immobilising bacteria for maximum biosorption and the underlying

mechanism are emphasised. On the basis of their biosorption potentials, the characteristics of cell wall components like peptidoglycan and the function of functional groups like carboxyl, amine, and phosphonate are discussed.

We analyse the binding mechanisms and the factors affecting the passive uptake of pollutants. There is a thorough explanation of isotherm and kinetic models as well as the significance of mechanistic modelling. For the efficient removal of metal, biomass must be modified chemically and genetically to increase biosorption ability. A packed column configuration is recommended for the continuous treatment of effluents, and the factors affecting its effectiveness are discussed. The chapter also emphasises the need to test biosorbents in actual applications because competition between solutes and water quality may impact biosorption effectiveness. As a result, this chapter examines the advancements and current state of biosorption technology and offers perspectives on this area of investigation[7].

As a consequence of human activity, enormous amounts of harmful metals are discharged into the environment every year. These releases might be intentional and strictly controlled, like industrial emissions, or they can be unintentional, like chemical spills or incorrect land disposal. Lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper, and nickel are toxic metals of concern. The mining, metallurgical, electronic, electroplating, chrome tanning, textile, metal finishing, fertiliser manufacturing, steel, and automotive sectors are the sources of these pollutants. Rapid industrialization and technological advancement have increased the amount of heavy metals discharged into the environment, endangering ecosystems and human health owing to their toxicity, accumulation in food chains, and natural persistence. Once they enter the environment, hazardous metal species are hard to track down, and they cause harm as they travel from one biological trophic layer to another. A challenge of the twenty-first century is managing heavy metal discharges and removing toxic heavy metals from water bodies.

The three types of heavy metal removal techniques employed in industrial effluents are physical, chemical, and biological. Precipitation, ion exchange, filtration, membrane and electrochemical technologies, reverse osmosis, electrodialysis, adsorption on activated carbon, etc. are examples of physicochemical methods that have high capital and operating costs and may also produce secondary wastes that pose treatment challenges. As a result, the development of alternative techniques known as bioremediation methods has received attention recently. Among the techniques used by these technologies is biosorption. The passive immobilisation of metals by live biomass is referred to as biosorption or bioadsorption. The capacity of biological materials to absorb heavy metals from wastewaters via metabolically mediated or physically chemical routes is known as biosorption. The main groups of biosorbents for the removal of metals are bacteria, fungi, algae, industrial and agricultural wastes, and various polysaccharide materials. The dissolved metal ions in diluted complex solutions can be successfully sequestered by these biosorbents. Because of its high efficacy, reduction of chemical and biological sludge, low operating cost, ability to regenerate biosorbents, and potential for metal recovery, the use of biological material in the removal of heavy metals from industrial effluents has gained importance recently[8].

Infectious Biosorbents

The most numerous and adaptable microbes, bacteria make up a significant portion of the overall live terrestrial biomass, which is estimated to weigh g. Certain microorganisms were discovered in the early s to a great capability for accumulating metallic elements. Since then, bacterial biomass-derived biosorbents have gained popularity because to their small size,

capacity for regulated growth, and adaptability to a variety of environmental conditions. In addition, microorganisms have easy access to low-cost nutrition supplies. Among bacteria, the genera Bacillus, Pseudomonas, Streptomyces, Micrococcus, and Escherichia coli are effective metal biosorbents. Basic facts on the use of bacterial biomass for metal biosorption are compiled.

Through interactions with chemical functional groups like carboxylate, amine, amide, imidazole, phosphate, thioether, hydroxyl, and other functional groups found in cell wall bio-polymers, metal ions in solution are adsorbed on to bacterial surfaces. Electrostatic attraction, complexation, ion exchange, covalent binding, van der Waal's forces, adsorption, and microprecipitation are a few of the processes that are combined during biosorption. Due to variations in cellular components, the degree of biosorption varies not only on the kind of metal ions present, but also on the bacterial species. In most cases, very brief contact periods are enough to achieve a metal-bacterial biomass stable state. This is so that biomass may be employed in wet cells or as fine powder, both of which have low mass transfer resistances. For the design of wastewater treatment systems, the quick kinetics seen with bacterial biomass is advantageous.

Bacterial Organisation

Most bacteria have cells that are between 0.5 and 1.0 m in diameter, however some are larger than this. Bacteria have a straightforward morphology; the three most prevalent forms are spherical or ovoid, rod, and spiral, while there is a wide range of shapes owing to variations in genetics and habitat. Bacteria's tiny size guarantees quick meta- bolic processes. A "typical" bacterial cell has a cell wall, a cell membrane, and a cytoplasmic matrix made up of a number of non-membrane-enclosed components. The Gramme stain is used to categorise bacteria as either Gram-positive or Gram-negative. This categorization separates bacteria into two primary categories based on the properties of their cell walls. A layer of peptidoglycan that fully envelops the cell and is abundant in carboxylate groups is present in both kinds of cell wall. The thickness of the peptidoglycan layer in gram-positive cells is ca. The Gram-negative peptidoglycan layer is just around nm thick. Three main elements make up the walls of Gram-positive bacteria: cytoplasm blended with peptidoglycan, and teichoic acids covalently bound to it. Compared to Gram-positive bacteria, Gram-negative bacteria have a more complicated envelope. It is made up of two membrane bilayers that differ from one another both chemically and functionally, sandwiched one on top of the other by a thin layer of peptidoglycan. The Gram-positive bacteria Teichoic Acids provide

Due to the existence of phosphodiester linkages between teichoic acid monomers, the cell wall has a general negative charge. The Gram-negative cell wall acquires a general negative charge as a result of the highly charged nature of lipopolysaccharides. The main contributors to the anionic character and metal-binding capacity of the cell wall are the peptidoglycan, phospholipids, and lipopolysaccharides of Gram-positive bacteria, as well as the peptidoglycan, teichoic acids, and teichuronic acids of Gram-negative bacteria. Extracellular polysaccharides can also bind metals, but they are also easily removed by simple mechanical disruption or chemical washing. Their availability depends on the bacterial species and growth conditions.

Bacterial cell walls include a variety of surface functional groups, with carboxyl often being the most acidic group in the bacterium. Cell wall ligands are protonated at low pH levels and fiercely fight with metals for binding. A higher pH might expose more ligands like amino and carboxyl groups, which would attract the metals' negative charges and so increase the amount of biosorption onto the cell surface. The S-layer and flagella are two examples of unique

structures seen in certain bacteria. A surface and paracrystal-line envelope called the S-layer is found in a number of bacterial and archaeal groups. Protein or glycoprotein monomers that can self-assemble into two-dimensional structures make up this layer. S-layers are related with lipopolysaccharides peptidoglycan of a Gram-positive cell or peptidoglycan of Gram-negative bacteria. Porosity ranges between and%, and pore sizes range from 2 to 8 nm. The metal binding industry may benefit from this property. The ability of this protein to reconstruct itself after being isolated from the cell is an essential property. This result makes it suitable for bioremediation. As a viable option for bioremediation of heavy metals in the field, S-layer proteins may play a role in the trapping of metallic ions in both live and dead cells.

Some bacteria have the ability to create capsules outside of their cell walls. They are highly hydrated, amorphous polymers of proteins and carbohydrates. Most capsules are made of polysaccharides, but some also include proteins or polypeptides, which are polymers of amino acids. The anthrax bacillus, Bacillus anthracis, is capable of producing polypeptide capsules with d-glutamic acid subunits. Depending on the particular organism, capsules may be hard or flexible, thick or thin. The capsule layer is referred to by a number of various names, including slime layer, glycocalyx, and EPS. Although capsules might include neutral polysaccharide, charged polysaccharide, or charged polypeptide, the majority of capsule polymers are acidic in nature. Metal binding depends on capsule positioning. Depending on the strain and the growth conditions, bacterial EPS has a diverse composition. Numerous pseudomonads, including Zoogloea ramigera, Rhizobium sp., Klebsiella sp., and Bacillus sp., have also been shown to synthesise EPS. EPS typically contains polysaccharides, proteins, and occasionally nucleic acids, lipids, or humic substances. Carboxyl, hydroxyl, and uronic acids are just a few examples of the many negatively charged functional groups that are abundant in EPSs. Through electrostatic interactions, these ligands enable EPS to grab metal ions and create a variety of complexes. EPSs have been suggested as a metal absorbent as a result of their extensive capacity to complexes heavy metals. Recent research by Yan et al. shown that the Bacillus sp. shown a fondness for copper.

Biosorption Mechanisms

The search for an effective biosorption process that should feature high metal selectivity and uptake requires localising the metal deposition site within the biosorption biomass, understanding the metal sequestering mechanism, as well as elucidating the relevant metal solution chemistry and chemical structure of the metal deposition site. The appealing aspect of bio sorption is a particular specificity of the biosorbent for divalent and multivalent heavy alloy cations. For different genera and even different mutant strains within a species, metal uptake can differ significantly. Important factors impacting a biosorbent's performance include the organism's nutritional status, physiological state, cell age, availability of micronutrients throughout growth, and ambient variables throughout the biosorption process. Biosorption also heavily depends on the metal's solution chemistry. There are numerous physicochemical mechanisms that can cause biosorption, and these mechanisms vary depending on the metal, its ionic form in solution, and the kind of active binding site that is in charge of sequestering the metal. Ion exchange, chelating, adsorption, and diffusion across cell walls and membranes are the key processes utilised in biosorption. These mechanisms vary depending on the species used, the location and manner of processing the biomass, and the solution chemistry[9].

Research is being conducted to determine if biosorption is a practical method for capturing and storing metals on a commercial scale. The removal of hazardous metals and the recovery of valuable metals are both possible via biosorption. Ion exchange is principally driven by the

biosorbent's attraction to the sorbate. Metals may be joined through complexation or electrostatic attraction. Less hydrophilic molecules have a lower affinity for the liquid phase and are thus more readily sorbed, which is where interactions between the solute and the solvent come into play. Electrochemically neutral metals are bound during adsorption and microprecipitation without a stoichiometric proportion of previously bound ions being released. The interaction between the solute and the solvent drives microprecipitation, while the affinity between the sorbent and the sorbate drives adsorption. A connection between biomass and metal is not required for microprecipitation.

Metal absorption is independent of cellular metabolism in the case of physicochemical interactions based on physical adsorption, ion exchange, and complexation between metal and functional groups of the cell surface. Electrostatic interactions, van der Waals forces, covalent bonding, or a mix of these processes are most likely involved in how a metal bonds to the cell surface. Electrostatic forces cause negatively charged bacterial cell wall constituents including carboxyl, hydroxyl, and phosphoryl groups to bind to metal cations. According to Tunali et al., Bacillus sp. Biosorbs lead and copper. Involve a method for exchanging ions. Since protons and metal cations compete for binding sites in the major ion exchange mechanism involved in biosorption, pH is the operating parameter that has the most impact on the process. The protonation and deprotonation of metal ion binding sites depends on pH, which also affects the sorbate's access to those sites. It is also possible to liberate metal ions from the binding site by reducing pH. This characteristic is employed to regenerate biosorbent and recover metal cations.

Methods for Metal Biosorption Studies

Negatively charged and widely distributed carboxyl groups play a key role in the binding of metal cations. According to Mishra and Doble, the carboxyl and amino groups were in charge of chromate's ability to bind. At pH 3, amine group’s protonated and attracted negatively charged chromate ions via electrostatic interaction, according to Kang et al. Potentiometric titrations may provide details on the variety and quantity of binding sites. Pseudomonas aeruginosa was titrated by Kang et al. who also calculated the pKa values of the accessible binding sites. Jian-hua et al. successfully correlated the metal uptake capacity with the quantity of acidic groups found on Bacillus cereus biomass as determined by potentiometric titrations.

FTIR may be used to roughly estimate the binding sites' makeup and their role in biosorption. The FTIR spectra of Cd2+ loaded and unloaded Aeromonas caviae were examined by Loukidou et al. The scientists were able to foresee the potential participation of amino, carbonyl, carboxyl, and phosphate groups in the biosorption of Cd2+ thanks to a number of band transformations. The presence of amide, carboxyl, and phosphate groups in Rhodococcus sp. biomass was verified by Cayllahua et al. using FTIR spectra. Biomass may be examined using energy dispersive X-ray to learn more about its chemical and elemental makeup. Using EDX, Tunali et al. determined that an ion-exchange process was involved in the biosorption of both Pb2+ and Cu2+ loaded Bacillus sp. In order to understand the chemical composition of bacterial cell-bound lanthanum, Kazy et al. performed X-ray diffraction research and validated the involvement of cellular carboxyl and phosphate groups in the binding of lanthanum by Pseudomonas biomass. SEM micrographs have aided researchers in analyzing cell surface morphology before and after biosorption.

CONCLUSION

The accumulation of heavy metals, including uranium (U), in the environment is a major problem due to their toxicity and potential impact on human health and the ecosystem.

Bioremediation, the use of microorganisms to remove heavy metals from the environment, has emerged as a promising solution to this problem. Several factors affect the ability of bacteria to accumulate uranium, including the type of bacteria, the concentration of uranium, the pH of the environment, and the presence of other metal ions. Understanding these factors is critical for developing effective bioremediation strategies. Research has shown that certain types of bacteria, including Pseudomonas, Bacillus, and Shewanella, are effective at accumulating uranium. The concentration of uranium in the environment also plays a role in accumulation, with higher concentrations leading to greater accumulation by bacteria. The pH of the environment also affects uranium accumulation, with slightly alkaline conditions generally being optimal.Furthermore, the presence of other metal ions in the environment can affect uranium accumulation by bacteria. For example, the presence of calcium, magnesium, and iron ions can compete with uranium for binding sites on bacterial surfaces and reduce its accumulation.

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CHAPTER 20

A BRIEF DISCUSSION ON BIOSORBENT'S

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ABSTRACT:

Biosorbents are natural or modified biomaterials that can selectively adsorb and remove pollutants from contaminated water and air. These materials have received considerable attention in recent years as a potential alternative to conventional technologies for pollution control, due to their high efficiency, low cost, and eco-friendliness. This review provides an overview of biosorbents, including their types, properties, and applications in environmental remediation. We discuss the mechanisms involved in biosorption, such as physical adsorption, ion exchange, and biospecific interactions, and the factors that affect the biosorption process, such as pH, temperature, and contact time. We also highlight the advantages and limitations of biosorbents compared to other conventional adsorbents, such as activated carbon and synthetic polymers, and the potential of combining biosorbents with other technologies, such as bioremediation and chemical treatment, to enhance the efficiency of pollutant removal.

KEYWORDS:

Biosorbents, Biomaterials, Microorganisms, Pollutants.

INTRODUCTION

Since protons and metal cations compete for binding sites in the major ion exchange mechanism involved in biosorption, pH is the operating parameter that has the most impact on the process. At solid-liquid interfaces, the various chemical species of a metal that arise at various pH levels will have varying charges and adsorbabilities. The assessment of biosorbent potential has often been reported to be complicated by metal precipitation in biosorption tests carried out at alkaline pH values. Ph determines the speciation and solubility of toxic metal ions and also affects the properties of the biomass. At lower pH levels, many metals exist as free hydrated species; when pH rises, hydroxides develop, and finally precipitation is possible. By protonating or deprotonating metal-binding sites, pH affects the amount of negative charge on a material's surface. The nature of each metal's chemical interactions with biomass may be connected to the varying pH sorption patterns for distinct heavy metal ions. The ideal pH will vary for various metal ion biosorption systems.

In the same pH range, cations and anions exhibit various sorption patterns on sorbent. According to Ma and Tobin, cation biosorption is at its peak at higher pH ranges whereas anions absorption is favoured at low pH with typical maximum biosorption in the range of 1-2. The surface characteristics of the biomass are primarily impacted by solution pH. It is important to note that the existence of various chemical groups on the surface of microbe biomass that are polar or anionic in nature, including as carboxyl, phosphate, amine, amino, hydroxyl, and sulfhydryl, is what allows the biomass to adsorb or chelate metal ions. Such

groups will contribute to the electrokinetic potential of the surface. Various isoelectric points are demonstrated by various microorganisms owing to the diverse chemical compositions of the cell wall. The total charge of the biomass surface will become positive at pH levels below the isoelectric point, whereas it will become negative at pH levels above the isoelectric point. As pH rises, the negative charge on the cell surface generally increases until all pertinent functional groups are deprotonated, which encourages electrochemical attraction and cat-ion adsorption. Furthermore, a more effective competition between cations and H+ for binding sites on bacteria may be the cause of the increased metal absorption with a rise in pH. Due to the protonation of functional groups at lower pH levels, anions should interact more potently with cells as the concentration of positive charges increases. Numerous publications examine how this component affects biosorption effectiveness by looking at things like zeta potential, electrostatic attraction, and the role of ion-exchange systems[1].

As the pH rises, metal ions in solution undergo hydrolysis. Each metal experiences hydrolysis to a different degree at various pH levels, but the typical process typically results in the development of hydroxylated monomeric species, followed by polymeric species, and then, after ageing, crystalline oxide precipitates. At solid-liquid interfaces, the different chemical species of a metal that arise with pH changes differ in charge and adsorbability. As a result, metal adsorption on surfaces is highly pH-dependent, and for each metal, there is a critical pH range where the amount of metal adsorbed increases significantly. Typically, this range is less than one pH unit.

Temperature

Biosorption by nonliving biomass is not significantly affected by the temperature. In contrast, because living cells' metabolism is temperature-dependent, changes in this parameter will have a significant impact on the biosorption processes. Since adsorption and ion exchange are exothermic reactions, the rate of these processes will rise as the temperature rises. However, at high temperatures, cell walls might sustain long-term harm, which is why a decline in metal uptake is seen. Most of the increase in uptake has been reported in the temperature range of 4–°C, whereas only a marginal increase is observed between and °C. Metal uptake is decreased greatly when temperature is raised beyond this amount. Since this situation is simple to reproduce, it is always preferable to perform and evaluate biosorption at room temperature[1].

Initial Metal Ion Concentration

A higher initial solute concentration causes a higher solute uptake, suggesting that it can affect biosorption. This happens because the initial mole ratio of the solute to the accessible surface area is low at lower initial solute concentrations; as a result, the fractional sorption becomes independent of the initial concentration. The removal of solute, however, is highly dependent upon the initial solute concentration because, at higher concentrations, the sites available for sorption become smaller relative to the moles of solute present.

Biosorbent's Initial Concentration

The amount of biosorption is significantly influenced by the dose of a biosorbent. Due to the increased surface area of the biosorbent, which in turn increases the number of binding sites, a rise in biomass content often results in an increase in the quantity of solute that is bio sorbbed. On the other hand, as the dosage of biosorbent is increased, the amount of biosorbed solute per unit weight of biosorbent decreases, which may be caused by the complex interactions of several factors. The fact that the available solute is inadequate to fully cover the available exchangeable sites on the bio sorbent at high sorbent doses is a significant issue

and often results in limited solute uptake. Because of the limited specific absorption, the interference between binding sites caused by higher biosorbent doses cannot be disregarded[2].

Presence of Competing Ions

Wastewaters typically include various metals. Multiple variables, including the quantity of metals vying for binding sites, metal concentration, and biosorbent dose, are expected to result in interactive effects when more than one metal is present in wastewater. Single-metal ion species in aqueous solutions have been the subject of numerous biosorption studies. The presence of additional co-ions has a substantial impact on metal absorption since they will compete with one another for binding sites with the cell wall and membrane's non-specific functional groups. As a result, it is often observed that metal uptake from mixed solutions is lower than that from single-species systems. Generally, metal absorption rises as the ionic radius of metal cation increases, with metals with larger ionic charge demonstrating better binding to biomass. Additionally, it is discovered that the concentrations of the other cations affect how much the metal uptake is reduced in the presence of those cations. In example, when the concentration of other cations rises, absorption of the metal further declines. Bueno et al. observed that the presence of co-ions, whether in binary or ternary com- binations, lowered the metal absorption when compared with the single-metal system. They noticed that the inhibition of lead uptake caused by copper ions was greater than the inhibition observed when copper and chrome ions were present together. In the presence of other metal ions in solution, chemical interactions between these species as well as with biomass may take place, resulting in competition for adsorption sites on the surface. As a result, the first component's "parking space" is less, and its uptake is diminished.

Binding of metal ions on biomaterials is one of the elements that influences a sorbent's inclinations for biosorption and is greatly influenced by the physicochemical characteristics of the metallic species. It has been reported that the metal removal increases with the increase in ionic radius, which follows the order Pb > Cu > Cr. The discrepancies in sorption affinities may also be related to changes in the electronegativity of the atoms, which likewise follows the pattern Pb > Cu > Cr. An further explanation for the considerable inhibition of lead absorption in the presence of copper and the mild impact of chromium on lead biosorption is that the affinity increases with increasing electronegativity or ionic radius. Uslu and Tanyol found that Pseudomonas putida's competitive biosorption capabilities for Pb and Cu ions were lower than those in noncompetitive circumstances.

Industrial effluent contains low atomic weight metal ions, such as Ca2+, Na+, and K+. According to the experimental findings, these metal ions have minimal impact on the biosorption of heavy metals, demonstrating little biomass affinity for the lighter ions. The biosorption of metal ions is also impacted by the presence of anions. Kapoor and Viraraghavan observed that biosorption capability reduced in the presence of ethylenediamine tetraacetate, sulfate, chloride, phosphate, carbonate, glutamate, citrate, and pyrophosphate. Anions in solution might create a complex. Metal biosorption capability would be greatly decreased by the metal ions. In general, as ligand concentrations rise, biosorption decreases.

The creation of Bacterial Biosorbents

The creation of stronger biosorbents and the planning of more effective biosorption processes are viable strategies that could improve the efficiency of heavy-metal biosorption. Biosorbent development might be performed by either isolating organisms with high capacity or high selectivity to heavy metals or by customising genetically engineered organisms rich in high-

affinity metal-binding proteins or polypeptides. Bae et al. discovered that the metalloregulatory protein, MerR, which displays high affinity and selectivity for mercury, was exploited for the development of microbial biosorbents specialised for mercury removal. Expression of mer operon genes encoding for cysteine-containing mercuric ion transport proteins on E. coli is extremely efficient biosorbents for heavy metal removal. In addi- tion, several other metal-binding proteins, such as metallothioneins, phytochelatins , and metal-binding pep- tides were also expressed on E. coli to create powerful biosorbents. The MerP protein is a target for the creation of genetically modified biosorbents . Kao et al. employed recombinant E. coli biosorbents with overexpression of MerP proteins for the biosorption of copper, nickel, and zinc from aqueous solutions. Deng et al. demon- strated biosorption by immobilized recombinant cells expressing human met- allothionein proteins. Recombinant strains of Staphylococcus xylosus and Staphylococcus carnosus with surface-exposed chimeric proteins containing polyhistidyl peptides were created by Samuelson et al. Both strains of staphylococci obtained increased nickel-binding capabilities owing to the introduction of H1 or H2 peptide into their surface proteins.

Modification of the cell wall can significantly change the binding of metal ions because the biosorption process primarily involves cell surface sequestration. A variety of approaches have been applied for cell wall modification of microbial cells in order to boost the metal-binding ability of biomass and to clarify the process of biosorption. Physical treatments include heating/boiling, freezing/thawing, drying, and lyophilization. The different chemical treatments used for biomass modification include washing biomass with detergents, cross-linking with organic solvents, and alkali or acid treatment. Pretreatments could change the surface properties/groups by exposing more metal-binding sites, removing or masking the groups, or both. For example, grafting of long polymer chains onto the biomass surface by direct grafting or polymerization of a monomer might add functional groups into the surface of biomass.

Heavy Metal Biosorption and Equilibrium Studies

The kind of procedure, which is thought of as a quick physical/chemical process, determines the pace of biosorption. Ion exchange, coordination, complexation, chelation, adsorption, and microprecipitation are a few of the passive accumulation processes that can all be grouped under the umbrella term "biosorption." In equilibrium, a certain relationship prevails between solute concentration in solution and adsorbed state. The adsorption equilibrium relationship at a certain temperature is known as an adsorption isotherm because the equilibrium concentrations depend on temperature. To correlate adsorption equilibria in heavy metals biosorption, a number of adsorption isotherms that were initially used for gas-phase adsorption have been adopted. The Freundlich, Langmuir, Redlich- Paterson, and Sips equations are a few examples of typical equilibria. The most popular equations are those using Freundlich and Langmuir. These isotherms for the removal of heavy metals from water and waste- water by biosorbents are detailed below[3]

DISCUSSION

The plot of log qe versus log Ce has a slope with the value of 1/n and an intercept magnitude of log KF. When Ce is equal to 1, log KF and log qe are interchangeable. However, in other cases when 1/n = 1, the value of KF depends on the units upon which qe and Ce are expressed. A Freundlich constant n between 1 and indicates favorable adsorption. A higher number of n denotes a more intense interaction between the heavy metal and the biosorbent, while 1/n = 1 denotes linear adsorption, which results in constant adsorption energies across

all sites. The Freundlich isotherm is very good at fitting data from highly heterogeneous sorbent systems and can fit almost all experimental adsorption-desorption data.

A 1/n value larger than unity shows the existence of a curved upward isotherm, commonly called as a solvent-affinity type isotherm. Within this type of isotherm, the marginal sorption energy increases with increasing surface concentration. Sorption of solute on any sorbent can occur either by physical bonding, ion exchange, complexation, chelation or through a combination of these interactions. In the first scenario of physical bonding, since the solute is lightly attached, it may simply be desorbed using pure water. Given the fact that miscellaneous functional groups such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphodiester groups can be present within the structure of the biosorbent, the mechanism of adsorption will not be restricted to physical bonding. As the interaction between sorbent and solute molecules is anticipated to be strong, many processes may be at play [4].

The most crucial quality of an adsorbent is its capacity for adsorption. The quantity of adsorbate absorbed by the adsorbent per unit mass of the adsorbent is its definition. A number of factors, including pore and ppaper size distribution, specific surface area, cation exchange capacity, pH, surface functional groups, and temperature, influence this variable.

As a point of caution, the adsorption equilibrium data at high concentrations cannot be predicted by the Freundlich equation. Furthermore, at extremely low concentrations, this equation is not simplified to a linear adsorption expression. However, researchers seldom experience this challenge, since modest doses are commonly utilised in most biosorption investigations.

Langmuir Isotherm

Another prominent model for modelling heavy metal sorption to biosorbents is the Langmuir model. The concentration of a medium above a solid surface at a constant temperature and the coverage of molecules on the solid surface are related by the Langmuir equation. This isotherm is based on three presumptions: that adsorption is restricted to monolayer coverage; that all surface sites are identical and can only support one adsorbed atom; and that a molecule's ability to adsorb on a specific site is independent of the occupancy of its nearby sites. These presumptions, along with a kinetic principle, allow us to formulate the Langmuir equation as follows:

In biosorption process, the saturation limit of particular biomass is determined by numerous parameters such as the number of sites in the biosorbent material, accessibility of the sites, chemical state of the sites , and affinity between site and metal . The amount to which a particular metal will occupy a given site in the case of covalent metal binding, if an occupied site is theoretically accessible, relies further on its binding strength and concentration in comparison to the metals currently occupying the site.

The decrease of KL value with an increase in temperature signifies the exothermicity of the adsorption process, while the opposite trend indicates that the process needs thermal energy , leading to chemisorption . At higher temperatures for physical adsorption, the bonding between heavy metals and the active sites of the biosorbent decreases as opposed to strengthening for chemisorption. The heat of adsorption may be used to evaluate whether the biosorption process is exothermic or endothermic. The Langmuir constant, KL, and temperature are related by an integrated Van't Hoff equation, where Ko is the adsorption equilibrium constant, Ea is the activation energy of adsorption/heat of adsorption, R is the gas constant, and T is the absolute temperature.

Isotherm Temkin

The Freundlich equation implies that the drop in heat of sorption as a function of temperature is logarithmic, although the Temkin isotherm assumes that it is linear. The Temkin isotherm has the form where b is the Temkin constant in proportion to heat of sorption and the Tem kin isotherm constant. Several experimental studies in chemisorption systems are correlated using this equation . Mondal et al. examined the biosorption of As, Fe, Mn, Cu, and Zn on Ralstonia eutropha. Temkin isotherms are unable to predict the biosorption equilibrium for a number of systems, including the biosorption of Ni by ureolytic mixed culture and the biosorption of Cr by exopolysaccharides from Lyngbya putealis. Since the basis of derivation for the Temkin equation involves simple assumptions, the complex phenomenon involved in liquid-phase adsorption is not taken into account by this equation. As a consequence, this equation is frequently not adequate for the description of experimental data in complicated systems. Dubinin–Radushkevich Equation.

It is very easy to interpret the sorption equilibria for organic compounds in porous solids using the Dubinin-Radushkevich equation. Due to the complexity of other factors like pH and ionic equilibria that are present in these systems, the DR equation is rarely applied to liquid-phase adsorption. Additionally, the bulk solution, where b is a constant and E0 is the solid characteristic energy towards a reference chemical, is often nonideal due to the interactions between the solute and the solvent. By taking into account the energetically nonuniform surface, this equation is capable of describing biosorption data as well. One of the finest characteristics of the DR equation resides in the fact that it is temperature dependent. All appropriate data must, in general, fall on the same curve, known as the characteristic curve, when the adsorption data at various temperatures are plotted as the logarithm of the quantity adsorbed versus the square of potential energy. This curve may subsequently be used as a preliminary "tool" to assess how well the DR equation expresses data on adsorption equilibria[5].

Model Brunauer-Emmer-Teller:

Bacterial Inoculants' Rhizosphere and Root Colonisation and Their Monitoring Techniques. Plants use their roots for a variety of purposes, such as anchoring, acquiring nutrients and water, and producing exudates with growth-regulatory qualities. The most active region of the soil matrix in terms of biology and chemistry is the root-soil interface, or rhizosphere. Rhizobacteria that promote plant growth are known to have an impact on plant health by preventing the spread of plant diseases or by directly stimulating plant growth in lab and greenhouse trials. Unfortunately, the outcomes on the ground have been less reliable. An essential phase in the relationship between the helpful bacteria and the host plant is the colonisation of the roots by the injected bacteria. But colonisation is a complicated phenomena that is impacted by a wide range of biotic and abiotic factors, some of which are just now becoming clear[6]. Independent of whether they are genetically modified or not, monitoring the fate and metabolic activity of microbial inoculants as well as their influence on the rhizosphere and soil microbial populations is required to ensure safe and reliable administration. The first and most important need for the efficient usage of PGPRs is the ongoing confirmation of strain identification and activity. For more efficient monitoring of inoculants strain after discharge into the soil, a mix of both conventional and molecular approaches must be improved. Future applications and evaluations of the effectiveness and reliability of microbial inoculants in crop production and protection will be influenced by recent advancements in methodologies for investigating rhizobacterial populations and detection and tracking systems of injected bacteria[7], [8].

CONCLUSION

Biosorption is an eco-friendly and cost-effective method for removing pollutants from water and wastewater. It involves the use of biosorbents, which are natural or synthetic materials that can bind to and remove pollutants from water. Biosorbents have several advantages over conventional treatment methods, including their low cost, high efficiency, and environmental friendliness. They can be easily obtained from various sources, such as agricultural waste, bacteria, fungi, and algae. Furthermore, they can be regenerated and reused, making them a sustainable option for water treatment.

Research has shown that biosorbents can effectively remove a wide range of pollutants, including heavy metals, dyes, pesticides, and pharmaceuticals, from water and wastewater. The performance of biosorbents depends on several factors, such as the nature of the pollutant, the type and properties of the biosorbent, and the operating conditions. The use of biosorbents has the potential to address some of the most pressing environmental and public health challenges associated with water pollution. However, there are still several challenges that need to be addressed, such as the optimization of operating conditions, the development of more efficient and selective biosorbents, and the scaling up of biosorption processes for industrial applications.

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CHAPTER 21

A COMPREHENSIVE STUDY ON RHIZOSPHERE AND RHIZOSPHERIC EFFECT

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ABSTRACT:

The rhizosphere is the region of soil surrounding plant roots where interactions between roots, microorganisms, and soil particles occur. This zone is essential for plant growth and development and plays a crucial role in the cycling of nutrients, water, and organic matter. The rhizospheric effect refers to the changes in soil properties and microbial communities that occur in the rhizosphere due to root exudates and other plant-associated factors. This review provides an overview of the rhizosphere and the rhizospheric effect, including the different types of microorganisms present in the rhizosphere, their interactions with plant roots, and the mechanisms involved in nutrient cycling and plant growth promotion. We also discuss the factors influencing the rhizospheric effect, such as plant species, soil type, and environmental conditions, and the potential of the rhizosphere as a tool for sustainable agriculture and environmental remediation. Furthermore, we highlight the recent advances in rhizosphere research, including the use of omics technologies to understand the complex interactions between plants, microbes, and soil, and their potential to improve crop productivity and soil health.

KEYWORDS:

Microbial, Plant Growth, Rhizosphere, Rhizospheric Effect.

INTRODUCTION

It is generally known that rhizosphere microbial populations are crucial for maintaining plant root health, nutrient absorption, and stress tolerance. These helpful microbes may play a crucial role in management strategies that provide achievable crop yields, which are yields that are limited only by the physical environment and genetic potential of the crop itself. In laboratory and greenhouse experiments, the idea of influencing crop rhizosphere microbial populations by inoculating beneficial bacteria to boost plant development has shown a lot of promise; nevertheless, outcomes have been inconsistent in the field. This technology is being developed because of the potential environmental advantages of this strategy, which include healthy sustainable management practises and a decrease in the usage of agricultural pesticides. The consistency of this technology in the market and its commercial development will be aided by recent advancements in our knowledge of the biological interactions that take place in the rhizosphere and the practical needs for microbial inoculant formulation and delivery systems[1].

Kloepper and Schroth initially used the term "plant growth-promoting rhizobacteria" to refer to soil bacteria that colonise plant roots after being inoculated onto seeds and promote plant development. The capacity to survive inoculation onto seed, proliferate in the spermosphere

in reaction to seed exudates, connect to the root surface, and colonise the growing root system are among the elements that make up the colonisation process. The difficulty of PGPR to colonise plant roots has often been cited as the reason for its field inefficiency. Only a few bacterial traits and genes have been found to be involved in root colonisation, but they include motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of certain cell surface components, ability to use certain components of root exudates, protein secretion, and more recently, the ability of the microbes to form biofilms and quorum sensing. Our understanding of the precise roles that each plays in the colonisation process is being aided by the generation of mutants altered in the expression of these traits. Using non-biased screening methodologies that depend on gene fusion technology, progress is being made in the discovery of novel, previously uncharacterized genes. These methods use in vitro expression technologies and reporter transposons to identify the genes expressed during colonization [2], [3].

Confocal laser scanning microscopy may be used to track the position of certain rhizobacteria on the root using molecular markers such fluorescent antibodies or green fluorescent protein. This method has also been used in conjunction with a rRNA-targeting probe to track the metabolism of certain rhizobacterial strains, and it has shown that bacteria near the root tip are the most active.

The capacity to outcompete native microorganisms found in the soil and rhizosphere of the growing plant is a crucial component of colonisation. Our inability to grow and characterise a variety of members of the rhizosphere community and to ascertain how that community changes with plant species, plant age, position on the root, and soil conditions has hampered our knowledge of the components involved in these interactions. It is now possible to describe the organisation of the rhizobacterial community using phenotypic and genotypic methods. Standard plating techniques on selective medium, community level physiological profiles utilising the BIOLOG system, phospholipid fatty acid, and fatty acid methyl ester profiling are phenotypic techniques that depend on the capacity to grow microorganisms. Direct extraction of DNA from soil, S-rRNA gene sequence analysis, bacterial artificial chromosomes, and expression cloning systems are the foundations of culture-independent molecular methods.

The complexity of the root environment, the diversity of rhizosphere microbial communities, and the significance of environmental and biological variables in shaping community structure are all becoming better understood as a result of this. These methods may also be used to assess the effects of PGPR vaccination on the rhizosphere community. To encourage plant development and safeguard the health of plants, various microbial inoculants are applied to plant seeds and seedling roots.

There are several known biotic and abiotic variables that affect how well injected bacteria function in the field. The survival, colonisation, and establishment of inoculants in the rhizosphere may be influenced by these parameters; however, in many instances, no evaluation has been conducted to identify the existence and colonisation of injected bacteria in the rhizosphere. This is largely because the imported strain lacks selection criteria to differentiate it from native bacteria. The development of inoculants with particular markers to be included for detection and colonisation in the rhizosphere and to measure their performance has been made possible by recent advancements in molecular methods. In order to guarantee that inoculants work more consistently and effectively in the field, the current chapter seeks to offer an overview of rhizosphere colonisation by rhizobacteria and techniques used to detect, identify, and monitor colonisation by injected bacteria in the root zone[4].

The Rhizosphere and Rhizospheric Effect

Hiltner was the first to use the word "rhizosphere" to refer to the area of soil where plant roots are active. The region with the greatest variety and activity of microbes is the rhizosphere. Living roots have a big impact on the soil between 0 and 2 mm below the surface. Thus, the longitudinal and radial gradients associated with growing roots, nutrient and water intake, exudation, and subsequent microbial proliferation are referred to as the rhizosphere. The formation of the macro- and microbiota's communities, exudate production, and root growth all depend on the rhizosphere stimulation of bacterial growth. The rhizospheric effect is a phenomenon that occurs surrounding the root as a result of the release of numerous organic chemicals by the roots. One of the most amazing metabolic traits of plant roots is their capacity to exude a wide variety of substances into the rhizosphere, with almost % of all photosynthetically fixed carbon being transported to the rhizosphere via root exudates.

Rhizosphere Colonisation

Historically, low- and high-molecular weight chemicals have been used to classify root exudates that plants discharge into the soil environment. Compounds with a high molecular weight include proteins, mucilage, and polysaccharides. The root cap, the principal cell wall between the epidermal layer and the sloughed root cap, and the epidermal cells all exude plant mucilages. During autolysis, lysates are released from roots. Additionally, microbial mucilages are released by rhizospheric microbes. Mucigel is the term used to describe a mixture of plant and microbial mucilages, microbial cells, and their products as well as related organic and mineral materials. Ethylene, sugars, amino acids, vitamins, polysaccharides, and enzymes are a few of the low-molecular organic substances emitted by plant roots. The notion that dietary resources have an impact on population dynamics and are important for niche colonisation and competition. Bacteria, fungus, yeasts, and protozoa make up the microbial community in and around roots. Bacterial communities in Root colonisers may include harmful, symbiotic, and bacteria that encourage plant development. The plant-beneficial microorganisms may be categorised as biofertilizers, phytostimulators, rhizoremediators, and biopesticides based on these activities. The molecular foundation of colonisation in these plant-microbe interactions is not well understood, despite how crucial they are to plant development. This is a major factor in why PGPR has had little success in real-world settings[5], [6].

A bacterium's colonisation of plant roots may be seen as an enrichment of the microorganisms most suited to a certain ecological niche. Rhizosphere colonisation is the process of the soil's surface area being populated by the root's activity. Rhizosphere colonisation is necessary for the use of microorganisms for beneficial reasons as well as being an essential initial stage in the pathogenesis of soil-borne pathogens. Numerous bacterial characteristics and particular genes influence colonisation, albeit few have been discovered. By colonising the root system and preventing the development of harmful rhizosphere microbes or inhibiting them, PGPR typically enhances plant growth. It is commonly accepted that efficient colonisation of the plant's rhizosphere by PGPR is necessary for biocontrol; nevertheless, this colonisation must be able to compete with native bacteria. Therefore, a biocontrol agent should be able to multiply and eventually colonise the plant root's surface.

An crucial phase in the relationship between helpful bacteria and the host plant is the colonisation of roots by injected bacteria. Root colonisation begins with seed colonisation. As the seed emerges and develops through the soil, microorganisms that have already colonised

the germination site might proliferate and colonise the root. Therefore, colonisation of the imbibing seed may be a precursor to future root colonisation. The capacity to effectively colonise a root surface is closely related to the competitive exclusion of harmful rhizosphere species. All of the disease-preventive processes that florescent pseudomonads have shown, in essence, are completely useless unless these bacteria can effectively colonise the root environment.

Root colonisation is Directly Involved in Competition for Root Niches and Bacterial Determinants

Significant carbon sinks include the rhizosphere and root surface. The zone's photosynthate allotment may reach %. As a result, there are several nutrient-rich areas along root surfaces that are favourable for a variety of microbes, including phytopathogens. A key mechanism by which PGPR shields plants from phytopathogens is competition for nutrients and niches. Genetic and environmental factors affect the amount and make-up of chemoattractants and antimicrobials released by plant roots. This suggests that the ability of PGPRs to effectively use a particular environment or to adjust to changing circumstances substantially influences their competency. In contrast to non-PGPR found in the rice rhizosphere, rice exudates produce enhanced chemotactic responses in endophytic bacteria. This suggests that PGPR may be especially suited to detect chemo attractants. The O-antigen chain of bacterial lipopolysaccharides, in particular, contributes to root colonisation.

However, as the O-antigenic side chain of Pseudomonas fluorescens WCS4 does not contribute to potato root attachment but the O-antigen chain of P. fluorescens PCL is engaged in tomato root colonisation, the significance of LPS in such colonisation may vary depending on the strain. The plant-beneficial endophytic bacterium P. fluorescens WCS7r does not colonise the tomato rhizoplane in part due to the O-antigenic component of LPS; yet, this bacterial determinant was implicated in the endophytic colonisation of the roots. Additionally, it has recently been shown that PGPR colonisation of plants is facilitated by rapid bacterial growth rates, the capacity to synthesise vitamin B1 and the ability to exude NADH dehydrogenases. Type IV pili, well recognised for its role in the adherence of animal and human pathogenic bacteria to eukaryotic cells, is another factor that affects bacteria's capacity to colonise roots. Endophytic bacteria like Azoarcus sp. colonise plants in part thanks to the type IV pili. Phase variation, a routine process for DNA rearrangements managed by site-specific recombinase, affects bacterial characteristics necessary for efficient root colonisation. Effective root colonisation in certain PGPR is dependent on their capacity to produce a site-specific recombinase. When a rhizosphere-competent P. fluorescens strain's site-specific recombinase gene was transferred to a rhizosphere-incompetent Pseudomonas strain, the Pseudomonas strain's capacity to colonise root tips improved.

Biofilms in the Rhizosphere

Biofilms are multicellular assemblages of bacteria that cling to environmental surfaces. Plant-associated bacteria interact with the tissue surfaces of the host and develop biofilm-like structures up to mature biofilms that are widespread. The characteristics of plant tissue's surface, Biofilm structure is significantly influenced by the availability of nutrients and water as well as the propensities of the colonising bacteria. In agricultural, industrial, and medicinal contexts, biofilms are very useful and display both positive and detrimental actions. Numerous root-associated pseudomonads have been investigated in-depth and are utilised as biocontrol agents or to aid in the development of host plants. Pseudomonads that promote plant development have been seen to intermittently colonise the surface of the roots, forming as minute biofilms along epidermal fissures. Pathogenic pseudomonads, however, produced

thick, confluent biofilms. Pseudomonad root biofilms have been said to come in a variety of sizes, from modest multicellular clusters to substantial biofilm networks.

It is well known that Azospirillum brasience and other species that are related to it may colonise the roots of cereals like wheat and maize. This free-living diazotrophic bacterium has been reported to promote plant growth by colonising root elongation zones and root hairs, which is followed by the development of biofilm. Rhizobia's capacity to produce root nodules and the development of biofilms in coiled root hairs are related. Rhizobia and Agrobacterium tumefaciens create thick biofilms on root surfaces that completely cover the epidermis, root hairs, and inorganic surfaces. Gram-positive bacterium Bacillus cereus grows in dense surface-associated populations, and a recent research has connected biocontrol to this species' capacity to form biofilms. It's likely that a number of processes that affect biocontrol activity also contribute to the development of biofilms.

The production of bio-films and colonisation both depend on a variety of microbial cell features, including adhesins, type IV pili, LPS, and flagella. Similar to this, numerous bacteria, including Pseudomonas aeruginosa and A. tumefaciens, are well-associated with the production of biofilms. The capacity of different pathogenic bacteria to create biofilms is now well-known and extensively characterised. In vascular pathogens such Xylella fasttidiosa, Xanthomonas campestris pv campestris, Pantoea stewartii sub sp. stewartii, Ralstonea solanacearum, and Clavibacter michiganensis, Ramey et al. have documented biofilm development. Quorum sensing is a cell-cell communication mechanism that is connected to several bacterial biofilm formation activities. On biotic and abiotic surfaces, the formation of biofilms by several different bacteria, including pathogenic, symbiotic, and free-living strains, has been shown more and more often. The nature and forms of biofilm that are generated depend on a number of parameters, such as surface chemistry, nutrition availability, and inherent bacterial abilities. Determining the role of biofilms to plant growth promotion by various rhizospheric bacteria, particularly root-associated free-living bacteria, is a crucial problem for future research efforts.

DISCUSSION

The intricacy of microbe-plant root interaction mechanisms has increased as a result of recent findings on quorum sensing and its alteration as a result of the presence of plant root exudates/metabolites. As bacterial populations grow in number, inter- and intra-species signal molecules are produced. These molecules help control the expression of genes that produce exoenzymes including pectin lyase, pectate lyase, polygalactouranase, cellulase, and protease as well as antibiotics. As a result, high inoculum density is often shown to be connected with rhizobacteria root colonisation.

Rhizobacteria's effectiveness and the Factors Affecting Root Colonisation

Specific bacterial features necessary for attachment and subsequent establishment are what predominantly affect bacterial root colonisation, although other abiotic and biotic variables are also significant. When an organism colonises a root, a variety of external criteria, such as water content, temperature, pH, soil types, composition of root exudates, and the presence of other microbes, must be used to validate the process. Another important factor affecting the variety of all microbes is plant species. In comparison to clay loam soil, the colonisation of a fluorescent Pseudomonas strain in the potato rhizosphere was shown to be ten times larger in a sandy loam soil. Predation and parasitism have a deleterious impact on bacterial root colonisation. The natural ants that live in the soil must compete with the inoculated bacteria for nutrition. Competition for nutrients may have an impact on rhizobacteria's ability to

synthesise antagonistic substances like antibiotics. Additionally, the production of antibiotics is crucial for the development of bacteria in the rhizosphere.

It's possible that different PGPR for biocontrol don't provide the same outcomes in the field as they do in vitro. The incapacity of PGPR to colonise plant roots is commonly linked to their inability to achieve the intended effects after seed/seedling inoculation. A number of qualities related to adaptability, tolerance, competition with native rhizospheric bacteria, and expression of root colonising features are significant in the complicated process of root colonisation. The fertility of the soil has often been decreased in various nations due to severe weather conditions, population pressures, land restrictions, and the demise of traditional soil management practises. These severe consequences will undoubtedly change the chemical, physical, and biological characteristics of the soil and thus impair microbial colonisation. Both the community they are introduced into and the native soil microbial populations may have an impact on biocontrol agents. Increasing populations of newly imported PGPR results in increased inhibition of Pathogens may be strengthened by altering a variety of field cultural practises. Application of organic material can be part of this.

A single biocontrol agent is only effective against a specific pathogen in a lab setting; it is not active against all pathogens that attack the host plant. This might be the cause of the patchy performance of field-tested biocontrol agents. Instead of large populations of a single organism, naturally occurring biocontrol arises from combinations of agents. Utilising PGPR strain combinations, greater suppression and improved consistency against several cucumber pathogens were reported. Co-inoculant incompatibility may sometimes occur, inhibiting both the target pathogens and one another as a result. Therefore, this is a crucial need for the effective generation of strain combinations. The inoculant strains' potential failure to survive and colonise the root is much more significant. The relationships between plant development stages and patterns of survival and efficacy, as well as attempts to separate injected PGPR from native microbial populations, have not been well examined. Therefore, many techniques are used to keep track of inoculant strains, both genetically engineered and unmodified. Below is a quick description of a few methods.

Microbial Inoculant Monitoring

A wide variety of monitoring techniques have been developed for the identification and measurement of microorganisms for different objectives. The three categories of monitoring techniques are molecular, direct, and microbiological. Here is a quick explanation of the popular techniques for keeping an eye on biocontrol agents.

Techniques for Microbiological Monitoring

These techniques, which are based on culture, are widely used to research and keep an eye on soil microorganisms, including those introduced into the soil system to see whether they survive and establish colonies on root surfaces and in bulk soil. The availability of selective media for target organ-isms to distinguish from local microorganisms is a fundamental necessity for such approaches. Based on physical traits, it might be difficult to differentiate injected organisms from natural populations. In order to differentiate with the local bacterial population, several publications have chosen the spontaneous mutant of the parent strain that is resistant to antibiotics like nalidixic acid and rifampicin. Prior to use, it is advisable to screen for antibiotic resistance among native populations that may thrive on certain medium. A quantitative technique based on CFU count and/or most likely number should be used when an appropriate method for detecting a target organism has been devised.

To use this strategy, you must first understand the features that are important for colonisation and then find mutants that have those qualities. Studies have examined topics such motility, the need for cell surface molecule production, O antigens of LPS found in outer membranes, prototrophy for amino acids and vitamin B1, and growth on seed and root exudates like organic acids and carbohydrates.

Cultivation-dependent techniques are often used to track the fate of inoculant strains in the rhizosphere of crop plants and nontarget plants. The use of rifampicin-resistant mutants of the PGPR strains is best for monitoring inoculants by selective plating since there are few native soil bacteria in the background that are rifampicin-resistant. Selective plating has previously been employed largely for strain confirmation, however it is now recognised that, although being quick and affordable, these methods are not sufficiently trustworthy for inoculant strain confirmation since spontaneous mutations may happen easily. Although these traditional methods have significant drawbacks, they are nonetheless feasible because of their reproducibility and simplicity in many circumstances, and they could provide valid cell counts.

Direct Monitoring Techniques

Direct monitoring techniques rely on the identification of the biological agent by the detection of a particular phenotypic trait, such as the emission of flourescence. A phenotypic trait that may be utilised to identify biological control/PGPR agents is bioluminescence. This method is based on the insertion of an exogenous reporter gene that codes for the bioluminescent proteins or enzymes.

The lux gene from the bacteria Vibrio fischeri and the gfp gene from the jellyfish Aequorea victoria are the most commonly mentioned reporter genes. Utilising optical detection techniques like flow cytometry, spectrofluorometry, or fluorescence microscopy, direct monitoring quantification is made possible. GFP markers with flow cytometry and the gfp/lux dual marker with flow cytometry and spectrofluorometry are often used by authors that use direct monitoring techniques for biological control agents in environmental samples to track P. flourescence.

The identification and counting of PGPR discharged during field inoculations have been emphasised as being crucial steps in determining whether or not they would survive in the field. The traditional methods for isolating and detecting bacteria in environmental samples include fluorescent-antibody and selective plating techniques.A laboratory test known as direct fluorescent antibody employs antibodies that have been fluorescently dyed to identify the presence of bacteria. Using fluorescently labelled antigen-specific antibodies, this approach allows simple antigen detection. DFA is seldom quantitative since its main objective is the detection of the antigen in a substrate or sample.

Using immunological approaches, microorganisms may be quantified and visualised in situ. They are founded on particular antibodies that are targeted towards bacterial antigens. The immunoflourescence colony staining technique is more informative than the conventional enzyme-linked immunosorbent test process because it combines quantification with visualisation in planta. Quantification also use immunomagnetic attraction. Immunofluorescence microscopy has been successfully utilised to identify Pseudomonas strains capable of colonising roots. The colonisation of roots of leguminous and nonleguminous plants after inoculation was investigated using monospecific polyclonal antisera produced against the bacteria Rhizobium leguminosarum bv. trifolii R, which was initially isolated from redclover nodules[7].

Molecular Monitoring Techniques

Lac Z and Lux Gene-Based Reporting Methods

Many of the benefits of traditional approaches may be achieved using luminescence-based methods, which also do not need laborious cell marking detection procedures in soil samples. Using mini-Tn5 delivery vectors, stable integration into the bacterial genome was made possible. Without the application of any selective markers, the technique allowed the identification of tagged Rhizobium meliloti in the presence of more than 5 CFU per plate. There are discrepancies between the marked strain and the wild-type strain in terms of growth rates or soil survival. A strong link between cell biomass and bioluminescence was also found in studies of the bioluminescent R. meliloti. The firefly luciferase tag-ging system is a quick, secure, and accurate way to find and count bacteria in the environment.

Root colonisation was less extensive in a mutant strain of P. fluorescens WCS5 with the Tn5lacZ mutation than in the natural type. The gene that codes for NADH dehydrogenase has a significant impact on root colonisation, as shown by Dekkers et al. The sss gene, which codes for a lambda integrase family site-specific recombinase that aids in adapting cells to rhizosphere conditions, is another gene necessary for effective colonisation. Additionally, it was proposed that a two-component mechanism including the genes colR and colS is crucial for P. fluorescens strain WCS5's capacity to colonise roots. According to a recent research by Miller et al., Pseudomonas putida needs the rpoS gene to colonise plant roots in a hostile environment. Rainey used a unique promoter trapping technique to identify as many genes that were triggered during root colonisation. The purB gene, which regulates rhizosphere colonisation in Pantoea agglomerans, has been described by Chauhan and Nautiyal.

The rod-shaped bacteria V. fischeri, which is found in marine species, has a collection of genes known as the lux operon. The 9-kb fragment known as the lux operon regulates bioluminescence by catalysing the activity of the luciferase enzyme. A group of genes known as the Lux operon code for the bacterial luciferin-luciferase system. Two genes are involved in controlling the operon, and five such genes have been shown to be active in the emission of visible light in V. fischeri. This gene set's transcription seems to be induced and inhibited by a number of external and internal stimuli, which results in either light emission or its suppression. Despite the fact that the lux operon encodes the enzymes required for the bacteria to light, autoinduction controls bioluminescence. A transcriptional promoter of the enzymes required for bioluminescence is known as an autoinducer. An autoinducer must be present in a certain concentration before the glow may happen[8].

Therefore, the organism must have large colony densities of V. fischeri for bioluminescence to develop. The scope and prospective applications of bacterial bioluminescence as a secure, quick, and sensitive sensor for a range of substances and metabolic processes have been substantially increased by the isolation of the lux genes and the capability to transfer these genes into prokaryotic and eukaryotic species. Before being planted in non-sterile Promix and natural soil, maize and lettuce seeds were treated with derivatives of all strains tagged with lux genes for biolumines- cence and resistance to kanamycin and rifampin. The number of newly introduced bacterial strains was determined on the roots by dilution plating on antibiotic medium and monitoring bio-luminescence.

A number of bacteria have successfully expressed the eukaryotic luciferase genes, including those from the luminous click beetle Pyrophorus plagiopthalamus and the firefly Photinus pyralis. The identical process involving d-luciferin, ATP, and O2 is catalysed by a monomeric enzyme that is encoded by each gene. Additionally, Bacillus subtilis experiments comparing the expression of the two different luciferases revealed that bacteria producing

bacterial luciferase had slower growth than the parental strain. When these bacteria compete with native bacteria, this characteristic could provide them a selection disadvantage. On the other hand, no discernible change in B. subtilis growth was seen when eukaryotic luciferases were expressed. Additionally, the measured luciferase activity were almost three times greater than those of the bacterial luciferase. Other requirements for tagging microorganisms include the need to guarantee stable inheritance of the engineered tag, prevent the risk of spreading the marker gene among ecosystem populations, prevent the gene from being overexpressed, and prevent the use of markers that confer antibiotic resistance.

CONCLUSION

The rhizosphere is a crucial interface between plant roots and the soil environment. It is a dynamic and complex microhabitat where various biotic and abiotic factors interact to shape the composition and activity of the microbial communities. The rhizospheric effect refers to the stimulation of microbial activity and diversity in the rhizosphere by plant roots. Research has shown that the rhizosphere plays a critical role in various ecosystem processes, including nutrient cycling, soil structure and stability, and plant growth and health. The interaction between plant roots and rhizospheric microbes can also influence plant defense mechanisms against pathogens and pests.

The use of rhizosphere and rhizospheric microbes has several potential applications in agriculture and biotechnology. For instance, the manipulation of rhizospheric microbial communities can enhance plant growth and health, improve nutrient uptake and use efficiency, and reduce the need for chemical fertilizers and pesticides. In addition, rhizospheric microbes have the potential to produce bioactive compounds with various applications in medicine, bioremediation, and industry. However, there are still several challenges that need to be addressed to fully harness the potential of rhizospheric microbes. These include the identification and characterization of key microbial players and their functions in the rhizosphere, the optimization of plant-microbe interactions, and the development of effective and sustainable strategies for microbial management.

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CHAPTER 22

IMPORTANCE OF PESTICIDE-SOIL MICROFLORA INTERACTIONS IN BIOREMEDIATION

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ABSTRACT:

Pesticides are widely used in modern agriculture to increase crop yield and protect against pests and diseases. However, their extensive use can lead to soil contamination, which poses a risk to the environment and human health. Bioremediation, the use of microorganisms to degrade or transform pollutants, is a promising strategy for the cleanup of pesticide-contaminated soils. The success of bioremediation largely depends on the interactions between pesticides and soil microflora, which play a critical role in the degradation and transformation of pesticides in soil. This review provides an overview of the importance of pesticide-soil microflora interactions in bioremediation, including the mechanisms involved in pesticide degradation and the factors that affect microbial activity, such as pH, temperature, and nutrient availability. We also discuss the challenges and limitations associated with bioremediation of pesticide-contaminated soils, such as the potential for secondary pollution and the need for effective monitoring and management strategies.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Using mini-TnS delivery vectors, stable integration into the bacterial genome was accomplished. Representative Gram-negative bacteria such Escherichia coli, R. meliloti, P. putida, and A. tume- faciens were tagged using the approach that was devised. Without the application of any selective markers, the technique allowed for the identification of tagged R. meliloti in the presence of more than 5 CFU per plate. There were no appreciable differences between the marked strain and the wild-type strain in terms of growth rates or soil survival. A strong link between cell biomass and bioluminescence was also found in studies of the bioluminescent R. meliloti. The firefly luciferase tagging technique is a simple, secure, and accurate way to find and count microorganisms in the environment.

Following their introduction, microorganisms go through a broad range of processes, including growth, physiological adaption, and transformation into nonculturable cells, physical dissemination, and gene transfer. Therefore, it is probable that using a single approach to discover microbes, assess their activity in the rhizosphere, and determine the danger involved would only give you half of the story. Both approaches culture-based and culture-independent have benefits and drawbacks of their own. It is recommended that the most useful method for monitoring microbial inoculant in bulk soil and the rhizosphere is a poly-phasic approach. It is thus vital to apply a variety of approaches for a thorough evaluation of the destiny and impact of released microbial inoculants/PGPR since the

situation may rely on interactions between microbes and plants as well as other environmental elements. For both genetically engineered and unmodified inoculants discharged into the rhizosphere or the wider environment, microscopy, cultivation-based, and molecular-based approaches should be developed.

We may anticipate the release of additional PGPR products as our knowledge of the intricate rhizosphere environment, the PGPR's mechanisms of action, and the practicalities of inoculant formulation and distribution expands. Our capacity to control the rhizosphere to improve the survival and competitiveness of these advantageous microorgan- gans will determine how successful these products are. Rhizosphere management calls for taking into account inoculant composition and distribution, as well as soil and crop cultural practises. One or more features related to plant growth promotion may be added to PGPR strains by genetic modification in order to improve colonisation and effectiveness. It is interesting to utilise multistrain PGPR inocula with established functions since they may improve field consistency. As an alternative, it is anticipated that plant growth-promoting microorganisms with a variety of desired features and environmental tolerance would provide better outcomes.

They provide the opportunity to handle various diseases, action mechanisms, and temporal or geographical variability. The use of molecular tools is improving our capacity to comprehend and manage the rhizosphere, and it will produce new goods with increased efficacy. Multiple strain-based inoculants, on the other hand, will need closer attention to be paid to their survival, colonisation, and efficacy in the root zone[1].

Importance of Pesticide-Soil Microflora Interactions in Bioremediation

The modern use of insecticides from various chemical families to increase agricultural productivity has resulted in their accumulation in soils to levels that directly and indirectly affect the activities of soil enzymes and the physiological traits of nontarget soil microflora, including rhizobacteria that promote plant growth, and as a result, the performance of crop plants. Bioremediation refers to a broad range of biological techniques that may be used to remove harmful compounds from the environment, including pesticides. The employment of microorganisms with the capacity to degrade materials is thought to be the most successful and economical method of cleaning up pesticide-contaminated areas among biological techniques. Insecticides are degraded or transformed by naturally occurring rhizosphere bacteria, which is the main emphasis of the current review.

The bulk of commercially significant crops are heavily infected during cultivation with insect pests such pod borers, aphids, jassids, and pod flies, which significantly reduce yields. In order to battle insect issues and thereby boost agro-ecosystem output, pesticides, including insecticides, are now sprayed to crops and soils, sometimes excessively or without consideration. After repeated application, a sizeable fraction of pesticides may accumulate in higher soil layers and have negative effects on the operation of ecologically and agronomically critical soil microflora as well as their variety. Consequently, very high pesticide concentrations may cause a considerable reduction in soil fertility.

By solubilizing insoluble phosphates, fixing atmospheric nitrogen and transporting it to plants, facilitating uptake of other plant nutrients, and synthesising siderophores and phytohormones, rhizospheric bacteria, which includes plant growth-promoting rhizobacteria, help plants grow. However, documented results have shown that insecticide concentrations above advised values negatively affect rhizobacteria's physiological traits as well as a variety of plant metabolic processes, resulting in losses in biomass, symbiotic traits, nutrient uptake, and quantity and quality of seeds. Biological approaches may be used to detoxify or eliminate

insecticides from soils in order to avoid such losses and decrease reliance on chemical inputs to soil.

Pesticides' Toxicity to Plants and Soil Microorganisms

The vital function that soil microbial community's play in the cycling of soil constituents has an impact on soil fertility and plant development. Agrochemicals, which are used in current agricultural practises to achieve the highest crop yields, have a significant impact on the beneficial microbial communities. Pesticides are any chemical used to prevent, eradicate, repel, or mitigate the effects of a pest, among other types of agrochemicals. However, certain microorganisms may tolerate or develop resistance to insecticides. If bacteria are genuinely susceptible, pesticides will disrupt their essential metabolic processes.

The sustainability of agricultural crops is impacted by the persistent and careless use of synthetic pesticides, which has become a serious danger to helpful soil bacteria. Globally, the bigger challenge is how to lessen or minimise the effects of pesticides so that agricultural yield may be retained despite any possible harm these chemicals may cause to microorganisms engaged in nutrient cycle. An effort is made to emphasise the effects of pesticides on soil microorganisms and agronomic crops in the section that follows[2].

Effects of insecticides on rhizobacteria and crops

Insecticides from a variety of chemical families are employed as seed and/or soil treatments in contemporary high-input agricultural practises to reduce losses brought on by insect pests. Following treatment, these pesticides build up in soils to unacceptable levels and influence soil enzyme activity and physiological traits of nontarget soil microbiota, either directly or indirectly, which reduces soil productivity. In a soil made of black clay, it was discovered that a combination of monocrotophos or quinalphos and cypermethrin exhibited additive, synergistic, and antagonistic effects on bacteria and fungi as well as dehydrogenase activity. Even at the highest level of gg1, application of monocrotophos, quinalphos, and cypermethrin at various rates either alone or in combination to soil significantly increased bacterial and fungal growth as well as soil dehydrogenase activity. However, when monocroto-phos or quinalphos were treated to the soil at the maximum rate together with cypermethrin, antagonistic interactions were more prominent for soil microbiota and dehydrogenase activity. On the other hand, synergistic or additive reactions occurred with the same mixture of insecticides at lower treatment rates. There are also certain PGPR strains that are resistant to insecticides. For instance, Nazarian and Mousawi discovered Pseudomonas and Flavobacterium strains that could withstand doses of guthion, methyl parathion, and dimethoate at 2, 4, and 8 g/L, respectively. These bacteria's resistance to such organophosphorus pesticides was presumably caused by the presence of plasmids that can express hydrolytic enzymes and degrade organophosphorous.

In a follow-up study, Vasileva and Ilieva conducted pot trials to determine the impact of pre-sowing treatment of seeds with insecticides carbodan ST at 1, 2, and 3 L/kg seeds and promet 0 SK at a dose of 3 L/kg seeds on lucerne's nodulating ability, nitrate reductase activity, and content of plastid pigments. It was discovered that the insecticides did not suppress nodulation; rather, the carbodan ST-treated plants' nodule counts and specific nodulation capacity rose by and 7%, respectively, in comparison to control. The pre-sowing treatment variations had roots that were 7% longer than the control. Nitrate reductase activity was elevated in the roots and leaves of the variants with carbodan at 2 and 3 L/0 kg seeds and promet, respectively. The total amount of plastid pigments rose in all versions treated with carbodan and decreased in the variant treated with promet compared to the untreated control[3].

Using 1.5 and 1 kg of active ingredient per hectare, respectively, Das et al. looked at the effects of phorate and carbofuran on the population and distribution of bacteria, actinomycetes, and fungi as well as the longevity of insecticidal residues in rice rhizosphere soils. In rhizosphere soils, pesticide application promoted the populations of bacteria, actinomycetes, and fungus. Compared to carbofuran, stimulation was stronger with phorate. However, neither pesticide significantly impacted Streptomyces or Nocardia in the soils of the rhizosphere. Increased populations of Bacillus, Escherichia, Flavobacterium, Micromionospora, Penicillium, Aspergillus, and Trichoderma were seen after treatment with phorate and carbofuran, respectively. On the other hand, the growth of Pseudomonas, Staphylococcus, Micrococcus, Klebsiella, Fusarium, Humicola, and Rhizopus as well as Staphylococcus, Micrococcus, Fusarium, Humicola, and Rhizopus under phorate stress was prevented. Similar to this, P-solubilizing activity of PSB was very little impacted by phorate at 0 and 0 g/mL, whereas IAA generation by phosphate-solubilizing bacteria from the genera Serratia, Pseudomonas, and Bacillus was significantly decreased.

Rodrguez and Toranzos examined the impact of lindane on microbial communities in soil that had previously been contaminated with different chemicals, including pesticides. Microbial communities were observed for days after soil microcosms were modified with 0 mg/kg of lindane. During the second week of the experiment, bacterial cell concentrations in the lindane-amended microcosms decreased by %. Overall, lindane had no influence on the genetic diversity and metabolic adaptability of these soils, showing that the bacterial communities can withstand the stress brought on by the addition of pesticides. In a separate study, pencycuron's effects on microbial biomass C, soil ergosterol content, and fluorescein diacetate-hydrolyzing activity varied depending on the field rate, 2FR, and FR concentrations used. Pencycuron-induced disruption at FR was revealed by changes in the microbial metabolic quotient and microbial respiration quotient. According to this research, the metabolically active microbial population was more repressed than the one that was dormant[4].

Glover-Amengor and Tetteh looked examined the impact of increasing rates of lindane, unden, dithane, and karate on garden eggs, okra, and tomatoes. All lindane rates reduced the yields of garden eggs. Lower lindane concentrations in tomatoes resulted in better yields, but higher concentrations decreased yields below the control. Despite modest yield increments, yields in okra were greater than the control at all lindane levels. Garden egg yields were most affected by unden spraying, followed by tomato yields, and okra yields the least. Although yields were greater in the control plots, in the garden egg and tomato treatments, increased concentrations of unden led to reduced yields. The ideal unden rate for tomato and garden egg was U. On okra, increasing the rate of unden had no discernible impact. Pesticide use decreased bacterial populations by -% while decreasing fungus populations in soil by -%. In general, dithane significantly reduced bacterial counts while karate significantly reduced fungal populations. Due to the lowest yield increase provided by any pesticide, lindane had no benefit over the others. In a groundnut field over the course of three years, Singh and Singh assessed the effects of diazinon, imidacloprid, and lindane treatments on ammonium, nitrate, and nitrite-nitrogen levels as well as nitrate reductase enzyme activity.

Imidacloprid and lindane were only given to seeds at recommended amounts whereas Diazinon was used for both soil and seed treatment. In both instances, diazinon residues lingered for days. Average diazinon half-lives were found to be between and days in terms of NO-N decrease and seed and soil reductase activity. Fox et al. came to the conclusion that organo-chlorine pesticides and other environmental contaminants cause a symbiotic phenotype of inhibited or delayed recruitment of rhizobia bacteria to host plant roots, fewer

root nodules produced, lower rates of nitrogenase activity, and a reduction in overall plant yield at the time of harvest. This was done through a study on the interaction of agrochemicals with crop plants. Additionally, according to Evans et al., omethoate was harmful to several Rhizobium strains when it came into direct contact with them when it was diluted via agar that had been seeded with these bacteria or combined with the bacteria in broth cultures[5].

DISCUSSION

Subterranean clover or lucerne seeds were inoculated with omethoate and a peat-based legume inoculant. On seedlings growing in sand pots over the course of three weeks, there were noticeably fewer nodules than on infected controls. By combining with omethoate, rhizobia counts were significantly decreased. In comparison to inoculated controls, seed preparation with omethoate before to inoculation had no impact on the number of nodules. In a different experiment, Evans et al. discovered that applying the Rhizobium meliloti inoculant to seeds that had already been treated with omethoate dramatically lowered the efficiency of the inoculation. In comparison to untreated plants, there was a 6 and% decrease in nodule counts and shoot mass per plant.

Unwise exploitation of natural resources has caused a significant amount of hazardous material pollution of land and water in many regions of the globe. As urbanisation and industrialisation increase, more contaminated areas are being found. Sites that are contaminated provide a major risk to both human health and the environment. There are several biological, physical, and chemical methods for cleaning up polluted land or water. Bioremediation is one of the most effective and cost-effective methods for addressing soil contamination issues. When toxins are present in soil, sediments, or water, bioremediation refers to the engineered use of biological agents, such as microorganisms or plants, to remove, neutralise, degrade, or transform the contaminants. Both in situ and ex situ bioremediation are possible. Since in situ bioremediation does not involve digging up soil, it is often used to degrade contaminants found in saturated soils and groundwater. Due to its cheap cost and use of harmless microflora to biodegrade dangerous compounds and their derivatives, this approach has a significant advantage over other bioremediation systems. Chemotaxis is a crucial characteristic in this technique because microbiological[6]

Therefore, in situ bioremediation may be made safer for breaking down dangerous substances by improving the chemotactic capabilities of cells. The use of in situ bioremediation has many advantages, including the simultaneous treatment of soil and groundwater and the lack of need for excavation of the contaminated soils, which makes it cost-effective. On the other hand, in situ bioremediation also has drawbacks: the process takes longer than other remedial techniques, and the effectiveness of the bacteria' capacity is dependent on seasonal variations and environmental conditions. Additionally, when contaminating compounds provide nutrition and energy for development, microbes function better. Microbes' capacity to breakdown contaminants decreases if these circumstances are unfavourable. The degradability of the contaminants must instead be accelerated by genetic manipulation of microorganisms, despite the fact that promoting indige- nous microflora is often recommended.

In order to promote microbial decomposition, ex situ bioremediation procedures include excavating polluted soils or pumping groundwater. Ex situ bioremediation is divided into two categories: solid-phase systems and slurry-phase systems, depending on the condition of the contamination to be removed. Problematic wastes and organic wastes may both be treated in solid phases. Composting, soil biopiles, and land farming are solid-phase soil remediation

techniques. Comparatively speaking to the other biological treatment techniques, slurry-phase bioremediation is a quick process. Slurry-phase bioremediation involves mixing polluted soil with water and other additives in a large container known as a bioreactor to maintain soil microorganism contact with pollutants. To provide the best environment for the microorganisms to break down the pollutants, nutrients and oxygen are introduced, and the environment in the bioreactor is monitored and managed. Water is removed from the solids when treatment is finished, and if they still contain pollutants, they are disposed of or given extra treatment[7].

Several mechanisms, such as volatilization into the air, sorption to soil components, movement in soils by runoff, leaching into soils, and upward movement in soils through capillary forces, take place during the dissipation of insecticides in the environment. The fundamental mechanism determining the dynamics of pesticide residues in the environment, including persistence in soils, is degradation, which is the main way for insecticide loss. Physical, chemical, and biological processes as well as living things both participate in the breakdown of pesticides. Enzymes that facilitate the breaking of molecules via various metabolic routes are what give pesticides their characteristic microbial breakdown, which is ultimately reliant on the viability, density, and enzymatic activity of soil microorganisms. In addition, the kinds and amounts of insecticides used or previously deposited in soils have a significant impact on the pace of deterioration. Because of their toxicity and widespread use in agricultural production systems, the microbial breakdown of insecticides, including lindane, chlorpyrifos, and monocrotophos, is covered in this section[8].

CONCLUSION

In conclusion, pesticide-soil microflora interactions play a crucial role in bioremediation processes. The use of pesticides can negatively impact soil microflora, which can in turn affect the biodegradation of pollutants. However, some microorganisms can also degrade pesticides, which can aid in the removal of both the pesticide and the pollutant.It is important to carefully consider the use of pesticides in bioremediation and to choose pesticides that are less harmful to soil microflora. In addition, incorporating beneficial microorganisms into the bioremediation process can enhance pollutant degradation and improve soil health. Further research is needed to fully understand the complex interactions between pesticides and soil microflora in bioremediation processes, as well as to develop more effective and sustainable bioremediation strategies.

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CHAPTER 23

ROLE OF ANAEROBIC BIODEGRADATION IN MICROBIAL TECHNOLOGY

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ABSTRACT:

Anaerobic biodegradation is the process by which microorganisms break down organic matter in the absence of oxygen. This process is essential for the degradation and recycling of organic matter in anoxic environments, such as groundwater, sediments, and the digestive tracts of animals. Anaerobic biodegradation plays a critical role in the global carbon cycle, and it has significant environmental applications, such as in the treatment of wastewater and the bioremediation of contaminated soils and sediments. This review provides an overview of anaerobic biodegradation, including the types of microorganisms involved, the metabolic pathways utilized, and the factors that affect the rate and efficiency of the process, such as temperature, pH, and nutrient availability. We also discuss the applications of anaerobic biodegradation in environmental remediation, including the use of anaerobic microbial communities to degrade organic pollutants and the potential of anaerobic bioreactors for wastewater treatment. Furthermore, we highlight the recent advances in anaerobic biodegradation research, such as the use of omics technologies to understand the complex microbial communities involved in the process and the potential of engineered anaerobic microorganisms for industrial applications.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

A persistent organic pollutant known as lindane, a broad-spectrum organochlorine pesticide, may infiltrate soil by direct application, the dumping of contaminated material, or wet/dry deposition from the atmosphere. Following application, HCHs are immediately absorbed by soil ppapers, released into the air or groundwater, or absorbed by crop plants together with polluted water. Because HCHs are tightly bound to soil organic matter, they are stationary in soils. However, lindane and other HCH isomers present a serious hazard to groundwater in the presence of low SOM and regular rainfall. Traditionally, three techniques have been documented for removing lindane from polluted sites: chemical degradation, physical adsorption, and bioremediation. Microwave irradiation, degradation with NaOH-modified sepiolite, and addition of hydrogen peroxide are some examples of chemical treatments. These procedures require caustic chemicals, hence they are not environmentally friendly.Physical approaches, in contrast, use thermal desorption and incineration, which provide enough deg- radation but need costly infrastructure. Additionally, they produce a lot of harmful gases. Although a very long process, biological therapies, such as the employment of bacteria sometimes referred to as "bioremediation," are a desirable choice because of their inherent environmental friendliness and cheap cost. In this situation, the isolation of

microorganisms with the ability to digest lindane has established certain strains that lindane and other HCH isomers may be degraded either aerobically or anaerobically. Some strains thrive when HCH is used as the only source of C and energy in the medium[1].

Pathway of Anaerobic Biodegradation

Clostridium sphenoides UQM0 was the first anaerobic lindane-degrading bacteria discovered. Other degrading bacteria, including taxa of Clostridium and Bacillus, were subsequently reported. However, there are discrepancies in the findings published for the breakdown of various HCH isomers, mostly due to the varying genera of microbes found in test soils and their level of resistance to pollutants. In contrast to - and -HCH, which were shown to be robust under both methanogenic and sulfate-reducing conditions, Haider and Jagnow observed that -HCH was considerably degraded.

Pathway of Aerobic Biodegradation

HCH only completely mineralsizes in aerobic environments. The four HCH isomers are being aerobically broken down by microbes in both mixed soil cultures and pure cultures. The bulk of research on lindane and other HCH isomers' aerobic degradative pathways has focused on Sphingomonas paucimobilis UT, a nalidixic acid-resistant mutant of Sphingomonas paucimobilis SS that eventually gives rise to mineralization. A putative ABC-type transporter system encoded by linKLMN is also crucial for the utilisation of -HCH in UT in addition to these catalytic enzymes. After UT's whole genome was sequenced, it was discovered that the lingenes needed to use the-HCH are spread over three large circular replicons, each measuring 3.5 Mb, 2 Kb, and 1 Kb. Other HCH-degrading bacterial strains included almost similar lin genes, and it has been proposed that plasmids and insertion sequence IS play a major role in lin gene spread. Two dehalogenases, LinA and LinB, were recently shown to have variations with just a few minor amino acid changes, and they demonstrated striking functional differences for the degradation of HCH isomers, showing these enzymes are still developing rapidly[2].

It was proposed that the combined application of plants and bacteria, also known as rhizoremediation, might speed up the elimination of persistent hazardous substances. Pesce and Wunderlin reported that sediment from a contaminated location on the Suquia River, Cordoba, Argentina, has been aerobically biodegraded of lindane by a consortium of bacteria, including Sphingobacterium spiritivorum, Ochrobactrum anthropi, Bosea thiooxidans, and S. paucimobilis. When exposed to lindane concentrations of 0.9, 0.7, and 0.2 mM, respectively, the bacterial consortia had initial lindane degradation rates of 4.3,.03, and.8 mM h1. Lindane mineralization was indicated by an increase in chloride content during aerobic biodegradation. During the first hour of biodegradation, a metabolite known as pentachlorocyclohexene developed. However, pure cultures of B. thiooxidans and S. pauci-mobilis decomposed lindane after three days of aerobic incubation, while Krishna and Philip evaluated carbofuran under different environmental conditions.The enhanced cultures often displayed varying degrees of deterioration and varied with various pesticides.

Compared to what was seen under aerobic conditions, degradation was more pronounced under facultative anaerobic conditions 3% of lindane was degraded by lindane-enriched cultures during an aerobic cometabolic process, compared to 5% during an anaerobic cometabolic process. In facultative anaerobic conditions, methyl parathion was only 1% degraded by methyl parathion-enriched cultures. Numerous intermediate metabolites were seen throughout the breakdown process, however some of them vanished after 4-6 weeks of incubation. It was interesting to see that the combination of pesticide-enriched cultures outperformed the single pesticide-enriched culture in terms of effectiveness and speed of

pesticide degradation. According to this research, consortia of bacterial cultures that can simultaneously detoxify the toxicity of many pesticides may provide an intriguing solution for repairing pesticide-contaminated landscapes. In addition to bacterial groups, soil is home to fungus, which are well-known for efficiently destroying lindane. For instance, the nonwhite-rot fungus Conidiobolus -1-, the white-rot fungi Cyathus bulleri and Phanerochaete sordid, and other fungus Pleurotus ostreatus have all been documented to degrade lindane by the release of specific enzymes.

Chlorpyrifos Biodegradation

Among other arthropod pests, chlorpyrifos is a broad-spectrum organophosphate pesticide that has insecticidal action against a variety of insects. Numerous studies have examined how diverse microbial communities, including those from the genera Flavobacterium and Escherichia Enterobacter, Arthrobacter, and Klebsiella, degraded chlorpyrifos in a variety of ecological settings.

Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, and Lactobacillus sakei were identified as the chlorpyrifos-degrading lactic acid bacteria isolated from kimchi fermentation in the presence of 0 mg chlorpyrifos L1. Chlorpyrifos served as the only source of C and P for all bacterial strains that were capable of decomposing substances. The investigated strains also destroyed other pesticides as coumaphos, diazinon, parathion, and methyl parathion. In another investigation, a bacterial strain M-1 that was isolated from sludge taken from a pesticide factory's wastewater treatment pool was subsequently identified as Paracoccus sp. by morphological and biochemical characteristics as well as S rDNA sequence analysis.monocrotophos in % in h. Strain M-1 utilised monocrotophos as its exclusive source of carbon. Monocrotophos was biodegraded by the action of constitutively produced cytosolic proteins, which were most active at pH 8 and C and exhibited maximal degradation rates of 0. mol mL-1 and 2. mol mL-1, respectively[3].

Although it was sensitive to high temperatures, the degrading enzyme was active in an alkaline environment. Within 7 days of incubation, species of Bacillus and Pseudomonas were shown to decompose % of dichlorvos, % of methyl parathion, and % of chlorpyrifos and phorate. Dichlorvos and phorate, on the other hand, were totally broken down by the end of the days. Phorate, dichlorvos, methyl parathion, chlorpyrifos, and methomyl were the sequence of microbial breakdown. When chlorpyrifos and methyl parathion residues were qualitatively analysed using gas chroma tography, it was discovered that inoculation samples had formed one unidentified metabolite whereas other insecticide-inoculated samples had not. Mallick et al. discovered that Flavobacterium sp. ATCC 1 isolated from diazinon-retreated rice fields rapidly degraded chlorpyrifos when it was added to a mineral salt medium or put to soil as the only source of carbon. An Arthrobacter sp. isolated from flooded soil and treated with methyl parathion also shown the capacity to degrade chlorpyrifos.

Additionally, Huang et al. investigated the degradation of chlorpyrifos in effluents from chickens and cows and found that in animal-derived lagoon effluents, chlorpyrifos was broken down by aerobic microbial activities. The denatured gradient gel electrophoresis of PCR-amplified S rRNA genes, used to analyse the microbial communities involved in the degradation process, revealed that a single band became dominant in effluents during chlorpyrifos degradation, indicating that a single aerobic bacterial population is responsible for chlorpyrifos degradation. Chlorpyrifos biodegradation is influenced by a variety of abiotic variables. The procedure heavily depends on the pH of the soil. Singh et al. investigated the impact of soil pH on the biodegradation of chlorpyrifos in Australian and UK soils and found that the cometabolic activities of soil microorganisms were responsible for the variation in

chlorpyrifos dissipation in UK soils between pH values of 4.7 and 8.4. In an Australian soil, there existed a large bacterial community that used chlorpyrifos as its only supply of carbon[4].

The spread of microbes that break down chlorpyrifos from Australian soil to UK soils was observed by PCR-denaturing gradient gel electrophoresis for the molecular fingerprinting of bacterial sRNA genes. The UK soils have a greater capacity to biodegrade chlorpyrifos. Additionally, only soils with a pH of less than 6.7 were able to continue to degrade things days after being inoculated. When soil pH rose from 4.3 to 7.0, the rate of deterioration in soils seeded with bacteria that break down chlorpyrifos increased, but there was no discernible difference between pH 7.0 and 8.4. Chlorpyrifos degraded more slowly in acidic soils than in neutral or alkaline soils. In a different research, Singh et al. revealed that an Enterobacter strain B- improved the biodegradation of chlorpyrifos. Based on an investigation of the strain's s rRNA, they discovered that this strain was most comparable to Enterobacter asburiae.

This strain converted chlorpyrifos into 3,5,6-trichloro-2-pyridinol and diethylthiophosphoric acid by hydrolyzing it as the only source of C and P. Because the gene encoding for this enzyme differed from the extensively researched organophosphate degradative gene in sequence, further research with B- showed that the strain featured a unique phosphotriesterase enzyme system. The authors came to the further conclusion that the application of strain B as a bioinoculant in soil that was polluted with chlorpyrifos resulted in a much faster rate of chlorpyrifos breakdown than was seen in noninoculated soils. Alcaligenes faecalis DSP3, which can break down TCP and chlorpyrifos, was identified by Yang et al. Additionally, Yang et al. were successful in cloning the mpd gene from a bacterium that breaks down chlorpyrifos and used it to bioremediate contaminated soils. By employing chlorpyrifos as the only source of carbon, six bacteria that break down chlorpyrifos were discovered. Their strain, YC-1, had the best capacity to degrade and was allegedly identified as belonging to the genus Stenotrophomonas. Within h, the strain YC-1 decomposed 0 mg/L of chlorpyrifos[5].

DISCUSSION

When the chlorpyrifos-degrading strain YC-1 was applied to fumigated and nonfumigated soils as a bioinoculant, the inoculated soils saw a faster rate of chlorpyrifos breakdown than the noninoculated control. Within days, the original concentration of 0 mg/kg of chlorpyrifos had entirely broken down. Chlorpyrifos degradation was significantly slower in non-fumigated control soils. The opd gene for chlorpyrifos breakdown, according to Guha et al., is found on plasmids as shown in Micrococcus sp. recovered from soil. The alkali lysis approach, however, did not reveal the presence of plasmids in the chlorpyrifos-degrading Stenotrophomonas strain YC-1, indicating that the opd gene was on the chromosome. But mpd and opd genes have also been discovered in various places on chromosomes and plasmids. For instance, Ajaz et al. proposed that the split position of the genes in Pseudomonas putida MAS-1 mediates the biodegradation of chlorpyrifos.

In a subsequent investigation, Li et al. recovered the chlorpyrifos-degrading bacteria strain Dsp-2 from a chlorpyrifos manufacturer's contaminated treatment system. By using molecular tools, morphological, physiological, and biochemical testing to identify this strain as Sphingomonas sp., it was discovered that it could hydrolyze chlorpyrifos to use it as the only source of carbon for development.It might also use phoxin and triazophos but not parathion, parathion-methyl, fenitrothion, or profenofos. The strain's capacity for bioremediation was next examined in a soil setting. When strain Dsp-2 was introduced to soil

that had received 0 mg kg1 of chlorpyrifos treatment, it demonstrated a faster rate of deterioration than control soils.Degradation was shown to be aided by the inoculum density, wetness, and mild pH. The chlorpyrifos-hydrolytic enzyme gene was discovered to have % similarity to mpd. Compared to the wild-type M6 mpd, mpd's hydrolytic efficiency for chlorpyrifos was substantially higher[6].

However, a variety of variables affect how chlorpyrifos degrades. Anwar et al. performed an experiment using Bacillus pumilus C2A1 for chlorpyrifos degradation to evaluate the effects of different culture conditions, such as pH, inoculum density, presence of additional carbon/nutrient sources, and pesticide concentration. Strain C2A1 used chlorpyrifos as the only source of C and energy and cometabolized it when glucose, yeast extract, and nutritional broth were present. The maximum chlorpyrifos degradation occurred at pH 8.5 and high inoculum density. However, the presence of additional nutrients further accelerated deterioration, perhaps as a result of the rapid growth on readily metabolised chemicals, which in turn accelerated breakdown. % TCP deterioration was also seen in the strain C2A1 after 8 days of incubation. In a related research, Lakshmi et al. found that three aerobic bacterial consortia, AC, BC, and DC, degraded chlorpyrifos more quickly in soil at days compared to days following growth in basal medium treated with mg chlorpyrifos L1. When grown alone in basal medium supplemented with mg of chlorpyrifos L1, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp., and Serratia marscecens decomposed chlorpyrifos by,,, and%, respectively, after days and,,, and%, respectively, after days. Chlorpyrifos was degraded by P. aeruginosa, which resulted in the formation of 3,5,6-trichloro-2-pyridinol, the main metabolite of chlorpyrifos degradation, which eventually decreased to insignificant levels. Thus, it is expected that this study will aid in overcoming the toxicity of chlorpyrifos in polluted environments, as would other related ones[7].

Monocrotophos

Gundi and Reddy conducted a laboratory study on the degradation of monocrotophos (1-methyl-2-methylcarbamoyl vinyl phosphate), a common organophosphorus pesticide, in two agricultural soils of India: a black vertisol and a red alfisol. The insecticide was administered under aerobic circumstances at % water-holding capacity at 4°C, and at two concentrations of 0 g g1 soil. Following first-order kinetics with rate constants of 0, the degradation of monocrotophos at both concentrations was quick, accounting for -% of the applied amount.MCP was degraded in soils by hydrolysis, which resulted in the production of N-methylacetoacetamide. At g g1 soil, even three applications of MCP did not increase degradation. N-methylacetoacetamide did, however, accumulate over time in soils that had previously had MCP treatment, or 7 g/g soil. MCP degradation was influenced by both biotic and abiotic elements. One research identified soil fungus that could break down MCP from different geographical locations. By employing MCP as a carbon and phosphorus source during an enrichment process, 25 strains were successfully isolated. The isolate M-4, known as Aspergillus oryzae ARIFCC, was chosen for further research based on the MCP tolerance capability shown in the gradient agar plate experiment. Under various cultures, the isolate's capacity to mineralize MCP was examined. It was discovered that the isolate has phosphatase activity. Using FTIR studies and high-performance thin layer chromatography, the degradation process' course was investigated. The findings point to the potential application of this organism for the bioaugmentation of MCP-contaminated soil and for the treatment of aqueous wastes. Light, moisture, and kind of water were shown to accelerate MCP degradation in soils.Rangaswamy and Venkateswarlu investigated how soil microorganisms degraded MCP and other organophosphates. They isolated six Bacillus strains and one

Azospirillum lipoferum isolate that were able to break down MCP. Chemical breakdown was less apparent and slower than microbial degradation[8].

Microorganisms capable of digesting MCP were isolated from 10 soil samples taken from Maharashtra by Bhadbhade as part of his study on the microbiological breakdown of MCP. In terms of isolates, % were from MCP-exposed soils, while % came from MCP-unexposed soils. This demonstrated the prevalence of and simplicity with which MCP-degrading bacteria may be isolated from exposed soils. Bacillus, Arthrobacter, Pseudomonas, and 2% of each of Planococcus and Stomatococcus were the taxa represented by the cultures. At the maximal starting dosage of 0 mg/L in synthetic medium, three cultures identified as Arthrobacter atrocyaneus, Bacillus megaterium, and Pseudomonas mendocina demonstrated -% degradation to MCP within h. The cultures could use MCP as the only source of carbon in synthetic medium and tolerated it up to a concentration of 2.0 mg/L. Under various environmental circumstances, such as pH values of 7.0-8.0, temperatures of -oC, MCP concentrations ranging from 0 to 0 mg/L, and an inoculum density of 8-9 cells/mL in synthetic medium under aerated growth conditions in h, the isolates demonstrated the greatest MCP degradation. MCP elimination varied between and %.

The synthesis of intermediary molecules, including one unidentified metabolite, methylamine, and volatile fatty acids like acetic acid or n-valeric acid, enabled the biodegradation of MCP to phosphates, ammonia, and carbon dioxide. The enzymes phosphatase and esterase, which were involved in the breakdown of MCP, were discovered to be present in the isolates. Based on the findings of the experiments, the microbial metabolic route for the breakdown of MCP has been postulated. In addition, it is now known that plasmids include the MCP degradation genes. In a related investigation, Subhas and Singh found that technical MCP could be degraded by Pseudomonas aeruginosa FB and Clavibacter michiganense subsp. insidiosum SBL up to.9 and.9%, respectively, while pure MCP could be degraded by and%, respectively, in shake-flask culture within h at °C. The ideal MCP concentration needed for proper development was zero ppm. The most favoured substrate was discovered to be tris-p-nitrophenyl phosphate, followed by paraoxon. Phosphotriesterase, which was localised on the membrane-bound portion of the ruptured cells, was the enzyme responsible for the breakdown of MCP. The phosphotriesterase-producing gene in P. aeruginosa FB was carried by a plasmid.

Insecticides generally have a negative impact on the metabolic processes of both crop plants and soil microorganisms. However, the harmful effects of pesticides on rhizobacteria's beneficial activities and plant development metrics are less severe when used at approved dosage rates. The capacity of soil microorganisms to fix nitrogen, the generation of phytohormones and other regulatory substances, photosynthesis, the buildup of dry biomass, and the overall nutritional status of crop plants have all been observed to decline at rates greater than those advised for use in the field. In order to detoxify persistent and excessive amounts of residual insecticides in soils, natural, affordable, and environmentally friendly bacteria with the ability to degrade pesticides may be a viable option[9].

CONCLUSION

In conclusion, anaerobic biodegradation is an important process in microbial technology that has the potential to remediate a wide range of organic pollutants. It involves the breakdown of organic compounds by microorganisms in the absence of oxygen, leading to the production of simpler and less harmful compounds. Anaerobic biodegradation can be used in a variety of settings, including wastewater treatment, bioremediation of contaminated soil and groundwater, and production of biogas. This process is often more effective and cost-efficient

than other methods of remediation, particularly for pollutants that are recalcitrant or difficult to treat. However, successful implementation of anaerobic biodegradation requires careful consideration of factors such as the type of organic compound, the characteristics of the microbial community, and environmental conditions such as temperature and pH. Monitoring and control of these factors are necessary to ensure efficient and effective biodegradation.

Further research is needed to better understand the mechanisms and limitations of anaerobic biodegradation, and to develop new strategies and technologies to optimize this process for the remediation of various organic pollutants. Overall, anaerobic biodegradation has great potential as a sustainable and environmentally-friendly solution for the treatment of organic contaminants.

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CHAPTER 24

A STUDY ON VITRO BACULOVIRUS PRODUCTS

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ABSTRACT:

Baculoviruses are insect-specific viruses that have been used for over three decades as a biopesticide and for the production of recombinant proteins in insect cells. The use of baculovirus expression vectors for protein production has several advantages, including high expression levels, post-translational modifications, and the ability to produce complex proteins. In recent years, advances in in vitro technologies have enabled the production of baculovirus products without the need for insect cell culture. This review provides an overview of in vitro baculovirus products, including the different types of products that can be produced, the methods used for their production, and their applications in research, biotechnology, and medicine. We also discuss the advantages and challenges associated with in vitro baculovirus product production, including the need for high-throughput and cost-effective methods, the potential for viral contamination, and the need for regulatory approval.

KEYWORDS:

Agriculture, Biosensors, Climate, Environment, Microbial.

INTRODUCTION

Since baculovirus insecticides are often extremely specific to their host insects and do not impact other arthropods, such as pest predators and parasitoids, they are excellent instruments in integrated pest control programmes. Additionally, they don't harm plants, animals, or the biosphere in any way. Over baculovirus products, most of which were created on insects raised on artificial diets, have been utilised against various insect pests across the globe. By using a baculovirus against wild populations of the insect host and collecting dead or dying larvae for later processing into a prepared product, there have been instances of large viral production in the field. Despite the widespread use of baculoviruses as biopesticides, compared to another biological insecticide based on the bacteria Bacillus thuringiensis Berliner, their utilisation is still relatively modest. Due to a number of technological restrictions, there are currently no programmes involving in vitro baculovirus commercial production, and further advancements in this field are urgently required.

Brazil has seen a decline in the use of the velvetbean caterpillar baculovirus over the last seven years as a result of soybean farmers changing their cultural practises. The slow killing rate of viral insecticides has prompted extensive study into creating quicker killing agents by genetic alterations, either by removing or introducing scorpion and spider toxin genes into their genomes. However, because to widespread public opposition to modified baclovirus genomes, these GMOs have not been used in actual practise. To increase the usage of these products globally, efficient public extension services and farmer education about use of biopesticides are critical[1].

At least a few viral families have been linked to insects and other arthropods. Due to its beneficial characteristics, including safety for the environment, people, other animals, plants, and natural enemies of pests, the Baculoviridae is the one that is most often examined with relation to its development as a microbial pesticide. Generally speaking, these viruses are quite selective, not impacting pest or other insect species. As a result, baculoviruses are excellent control agents for integrated pest management programmes in agriculture, forestry, and pastures. In the s and s, it was encouraged to use these substances as microbial insecticides.

Additionally, baculoviruses have shown to be very useful tools in biotechnology. One of the most popular techniques for the regular generation of recombinant proteins is the baculovirus-insect cell expression system. Recently, bacu- loviruses have shown they can provide perfect vectors for a range of mam- malian cell lines, making them viable candidates for gene therapy. Insects from the orders Lepidoptera, Hymenoptera, and Diptera are the most common arthropods that are susceptible to baculoviruses, which are a broad and varied group of viruses. Invertebrate-isolated baculoviruses have been reported in the literature in numbers greater than zero. Insect populations naturally harbour these viruses, which are often given the name of the first host from which they were isolated. They have been extensively employed as bioinsecticides for the control of several agricultural and forest pests due to their high virulence, specificity to insects, and environmental stability. Several of these viruses have been used as biological pesticide substitutes to control insects.

Baculoviruses are circular, covalently closed, double-stranded DNA genomes that range from to 0 kbp in length and encode for 0-0 proteins. They reproduce in the nucleus of infected host cells. More than baculoviruses have had their genomes sequenced, and many of them have since been examined and published. These viruses are part of the family Baculoviridae, which is presently split into four genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus, and Deltabaculovirus based on phylogenetic evidence and molecular traits. The 9th International Committee on Taxonomy of Viruses Report has suggested this categorization of baculoviruses. Lepidopteran NPVs may be divided into groups I and II for further classification. The existence of distinct envelope fusion proteins, GP and F, encoded by viruses from each group, has been linked to this split. In contrast to delta- and gammabaculoviruses, which typically contain a single nucleocapsid per ODV, alphabaculovirus virions are classified as single or multiple based on the number of nucleocapsids per ODV[2].

Baculoviruses come in two phenotypes, occlusion-derived virus and budded virus, both of which contain the same genetic material and have a same nucleocapsid structure. These virions are created at various periods and cell sites over the course of an infection. Additionally, they vary in terms of the makeup of their viral membranes, some of the virus-derived proteins they contain, and their modes of entrance into the host cell. The fusion protein GP or a different unrelated protein known as the F protein, which promotes systemic infection, are needed by BVs because they are formed in the late stages of infection and get their envelope from the cell membrane. Although a number of other proteins are solely linked to ODV, this protein generates structures at one end of the budded virus ppaper termed peplomers, which are absent in ODVs. The initial infection of midgut cells in insect larvae requires the presence of a number of ODV envelope proteins, while others are ODV components whose precise position and function are yet unknown. When nucleocapsids are wrapped within the nucleus and then occluded in a protein crystal structure to form the occlusion bodies, which are formed in the very late phase of the infection, ODVs are created.

ODVs initiate primary infection in the midgut throughout the baculovirus life cycle and are necessary for horizontal transmission of baculoviruses across insect hosts. These virions, which are produced from the nuclear membrane of the insect cell, get occluded in a protein matrix relatively late after infection, creating paracrystal-line structures known as occlusion bodies. The majority of the proteins in the occlusion bodies, polyhedrin in NPVs and granulin in GVs, are very stable and help viruses survive and spread in the environment. BVs may spread from cell to cell both within the insect and in a cell culture, and they are very contagious for insect cells. These virions have a different envelope from ODV, which promotes systemic infection. Through the plasma membrane, they buddingly obtain their envelopes[3].

When a vulnerable host consumes OBs that have been dropped on foliage by an earlier infected host, hundreds of ODVs are released into the stomach, starting the viral life cycle. The alkaline environment in the host midgut dissolves the crystalline polyhedron matrix around the ODVs. After being released, the ODVs cross the peritrophic membrane, adhere to the microvilli, and start a primary infection of the midgut's mature columnar epithelial cells. These cells create a budded virus that starts secondary infections and spreads throughout the host. The production of BV and ODV is caused by the release of the nucleocapsids from the endosomes and their transport to the nucleus, where viral transcription, DNA replication, and the assembly of progeny nucleocapsids take place. The majority of the nucleocapsids stay in the nucleus and occlude in a protein matrix to form OBs during the last stage of infection. When an infected insect dies and disintegrates or liquefies, progeny OBs are released, which then start a new cycle of infection in additional hosts. The occlusion bodies may be dispersed more easily if the terminally afflicted insect moves to a higher height on the plant branch.

A large family of double-stranded DNA viruses are known as baculoviruses. They only affect arthropods; they do not spread to plants, vertebrates, or microbes. Despite not reproducing, they may infiltrate animal cells under certain circumstances. Due to this surprising characteristic, baculoviruses have become an important tool for research on the transitory expression of foreign genes under the control of vertebrate promoters included into the baculovirus genome[4].

Since baculoviruses are one of the most adaptable tools for genetic engineering, they have attracted a lot of interest in molecular biology laboratories. The Autographa californica nucleopolyhedrovirus is the most extensively researched baculovirus. Our present understanding of the biology of AcMNPV is, in great part, a result of the advancements made with regard to expression vectors based on baculoviruses. The following are some of the advantages this method of foreign gene expression has over other methods:

a. It is usually possible to express more than one foreign gene;
b. The baculovirus genome can accommodate large pieces of foreign DNA;
c. Insertion of particular signal sequences in front of a foreign gene often results in export of the gene product outside of the infected cell.

AcMNPV's circular DNA genome is encircled by a little basic protein that balances the DNA's negative charge. A nucleocapsid of proteins surrounds this structure to safeguard it. A membranous envelope encasing one or more nucleocapsids makes up virions. In its bare form, genomic circular DNA is contagious. The two physically different but genetically identical viral types are created at various times after infection, as was already explained. The matrix surrounding the occlusion bodies, which are mostly made of the structural protein polyhedrin, contains several occlusion-derived virions. It should be emphasised that while polyhedrin is created in significant amounts, it is not necessary for the virus to spread from

cell to cell. As long as the environment is favourable, protected viruses may persist in polyhedra, which are rather stable. They can be seen with a light microscope because they are big enough. Polyhedra resemble transparent, asymmetric salt crystals when magnified by a factor of 1[5].

In most cases, recombinant baculoviruses are created in two phases. An initial baculovirus transfer vector contains a heterologous gene. A bacterial replicon comprising a multicopy plasmid, a selection marker gene, promoter and terminator sections, flanking baculovirus sequences from a non-essential locus, and a multiple cloning site downstream from a viral promoter make up the vector. The promoters and the surrounding DNA often come from one of the very late genes, either polyhedrin or p, when the creation of a recombinant protein is needed for commercial purposes. The latter is a different viral gene that codes for a protein that is synthesised in significant amounts relatively late in the infection. It makes up the majority of the fibrillar structures that build up in the cytoplasm and nucleus of infected cells. Weaker late but not very late or early promoters are sometimes desired for particular applications, such as for earlier strengthening of a baculovirus' insecticidal characteristics.

DISCUSSION

The second stage of recombinant engineering in traditional techniques of recombinant building is homologous recombination in insect cells. Introduced into insect cells, the baculovirus transfer vector carrying both genomic viral DNA and foreign DNA undergoes recombination to produce a recombinant virus with an incorporated heterologous gene. Recent years have seen significant advancements in recombinant selection techniques over traditional ones. The creation of recombinant baculoviruses is made simpler by linearization of the baculovirus genome at one or more sites. When compared to preparations of circular DNA, the infectivity of linear baculovirus DNA is significantly reduced. Recombinant viruses were produced at a frequency of around% when a special restriction site was added to the AcMNPV genome, allowing for linearization near the polyhedrin gene. It should be noted that circularization of the genome occurs as a consequence of recombination between linear genomic DNA and a transfer vector. Since there are fewer nonrecombinants arising from linear DNA in the background, even if the titer of recombinants per transfection is equivalent to that of the typical cotransfections with circular genomic DNA, the proportion of recombinants is much higher. The proportion of recombinant viruses decreased to virtually 0% when further improvements to the aforementioned technique were made.

The Bac-to-Bac expression technique is often used in labs that produce recombinant proteins to create baculovirus recombinants. The main processes of recombinant construction are shown in the schematic in Fig. 2. A bacmid is a bacterial plasmid with a low copy number that has been designed to include the whole baculovirus genome. The target gene is cloned onto a different little plasmid that is located downstream of the polyhedrin promoter. The gene of interest is flanked by two transposable elements on this plasmid, along with a gene for gentamycin resistance. To convert certain bacterial strains that have the baculovirus genome, the donor plasmid is utilised. These bacteria also have a plasmid that codes for the transposase enzyme, which facilitates the transposition of transposable elements from the donor plasmid to the viral genome. Because the viral genome contains an extra LacZ marker gene, the bacmid having the desired gene is thus acquired and may be visually picked. Insect cells are transfected using recombinant bacmid preparations from bacteria once the existence of the desired gene has been confirmed in the baculovirus genome. Within two to three days of transfection, a viable recombinant baculovirus ought to be budding into the culture medium.

There are a few hundred insect cell lines that may be employed for baculovirus in vitro propagation. Spodoptera frugiperda and Trichoplusia ni, two parental species, provided a few that aid in AcMNPV proliferation. Sf9 is the most popular and grows best in sus- pension. Viral proliferation has also been achieved using BTI-Tn5B1-4, often known as High Five cells, which are generated from T. ni. Lymantria dispar nucleopolyhedrosis virus, Helicoverpa zea nucleopolyhedrosis virus, Bombyx mori nucleopolyhedrovirus, Anticarsia gemmatalis nucleopolyhedrovirus, and a few additional baculoviruses may all be propagated using the cell lines that are now available[6].

For the generation of glycoproteins having therapeutic potential for both humans and animals, the baculovirus expression method is often utilised. These proteins' posttranslational changes largely mirror those seen in mammalian cells. However, compared to insect cells, the N-glycosylation of proteins in mammalian cells is more complicated. In contrast to sialic acid-terminated glycans in vertebrate cells, the latter produces N-glycans with terminal mannose residues. Most of the time, the level and type of glycosylation present in insect cells is adequate to preserve the biological functions of glycoproteins, and as a result, these insect-derived glycoproteins meet the criteria for possible therapeutic agents. It is feasible to employ "humanised" insect cell lines, which have been genetically modified to create the necessary vertebrate-type complex N-glycans with terminal sialic acids, under exceptional circumstances when the importance of glycan chains in the maintenance of biological activity is extremely high.

The only method currently used for the commercial production of baculoviruses is in vivo, either by directly exposing the host insect to the virus in the field and harvesting sick or dead larvae, or by raising the target insect in the lab on an artificial diet and contaminating the diet with a baculovirus before harvesting the virus-killed insects. In several nations, the latter approach is the one that is most often employed to create baculoviruses. The Anticarsia gemmatalis Alphabaculovirus has been successfully produced using both techniques in Brazil. Due to the dependance on the leaves of the host plant for viral inoculation, commercial production of baculoviruses of particular insects is often too difficult or impossible under laboratory settings. This is because some insects do not have access to artificial meals. However, field manufacturing of baculovirus agents is feasible and produces lower-priced goods[7].

However, when the liquefaction of the insect body is severe, as it is, for example, in larvae infected with Spodoptera spp. baculoviruses, making it almost impossible to gather dead larvae, field production becomes challenging. In this situation, live larvae must be gathered just before they pass away, before the body has burst. However, compared to dead larvae, these larvae may not be as virus-filled. It is well known that two viral enzymes, chitinase and cathepsin, play a crucial role in the Lepidoptera species' shared cuticle disintegration and liquefaction processes. It is feasible to locate a few naturally occurring isolates of the same baculovirus that are devoid of these enzymes, enabling field and laboratory production. To illustrate the intricacies of both manufacturing processes, the commercial field and laboratory manufacture of the AgMNPV are addressed in Sects. and.

In addition to being a procedure that can be controlled, is sterile, and produces a very pure product yield, baculovirus generation in insect cell cultures has benefits over in vivo multiplication. The development of baculoviruses for agricultural pest management must be effective, economical, and provide a highly pathogenic end product for the insect in question. However, there is a significant restriction on in vitro production since the virus loses virulence after several passages in cell culture due to genetic changes. Production of occlusion-derived virions is not essential for the virus to survive in laboratory culture. In cell

culture, the form of the virus that is employed for cell-to-cell transmission is the budded viral ppaper. The GP is the primary protein in the BV ppaper. This glycoprotein is necessary for viral budding during infection and is in charge of allowing the virus to enter the next host cell. Temperature, pH, dissolved oxygen content, osmolality, and nutritional composition of the culture medium are among the culture parameters known to affect baculovirus infection of lepidopteran cells. The majority of lepidopteran cells multiply best at temperatures between and °C with an ideal pH of 6.2. Insect cells are easier to cultivate, are more resistant to osmolality and by-product concentration, and exhibit greater expression levels when infected with recombinant baculoviruses than mammalian cells[8].

From a commercial standpoint, in vitro generation is still a crucial prerequisite for the use of baculoviruses as pesticides. The transition from the parental, many polyhedra per cell phenotype to the few polyhedra per cell phenotype is one of the viral passage's most significant impacts. The decreased occlusion and loss of virulence of the occluded virus are two major issues related to the passage effect. The k fp locus is located in a particular area of the Few Polyhedra mutants that is often mutated. For the creation of polyhedra and to prevent virion occlusion, this gene produces a -kDa protein. The development of Defective Interfering Ppapers is a different sort of mutant produced during the serial passage of baculoviruses. These mutants often have substantial deletions in their genomes and are unable to reproduce in the host cell without the assistance of a helper virus.

The need for a highly prolific insect cell line and a highly productive culture medium presents another difficulty for the in vitro generation of baculoviruses. There are several cell lines that may be used for manufacturing that come from different sources and have a range of growth and production properties. For a certain viral isolate-cell line combination, careful media formulation or screening is required since the yields of polyhedra may be significantly impacted by various media. A fresh approach to in vitro manufacture has just been put forward and is based on variations of the Many Polyhedra. These clones were chosen after the virus had passed through many cell culture passages using the plaque assay technique. In theory, MPs may compete with the population of Few Polyhedra mutants cultivated in cell culture since they retain wild-type characteristics including the creation of numerous polyhedra in the cell nucleus and a high titer of the budded virus. An important requirement for process optimisation of in vitro baculovirus production is the investigation of factors associated with loss of genetic stability and the use of new approaches such as isolation of more stable variants and the reduction of the cost of cell culture medium components.

Other publications have been published on the status of viral utili- zation against insect pests of agricultural, forest, and vegetable production systems since Moscardi's thorough assessment on use of baculoviruses for control of Lepidoptera. The most significant programmes from across the globe are the subject of this chapter, with special attention paid to factors that either promote or restrict the use of these agents in IPM programmes. As a case study, the usage of the AgMNPV in Brazil is discussed to show how a very successful programme suffered a significant setback over the previous six years. The AgMNPV is the biological product that is most often employed in Latin America to control A. gemmatalis in soybeans. During the growing season in Brazil, this virus was applied to around 2.0 million hectares of land, or about % of the total area utilised to raise soybeans there. Additionally, Bolivia, Paraguay, Argentina, Colombia, and Mexico have all utilised it[9].

The poplar moth virus, Condylorrhiza vesti- gialis, has a nucleopolyhedrosis that is now employed in Brazil. This virus was created on insects that were fed an artificial diet while being raised. The treatment of 2,0 ha/year, which represents the infected area among 5,0 ha of poplar plantations in south Brazil, is the main goal of its use. By the initiative of the

International Potato Centre, a granulovirus has been developed to suppress larval populations of the potato tuber moth, Phthorimaea operculella, in field and stored potatoes, in Peru. Ecuador, Colombia, and Bolivia have also utilised this virus. Currently, the Erinnyis ello GV in Colombia, another baculovirus utilised in Latin America, has taken the place of chemical pesticides in areas where the bug is endemic. Since there are no publications in the literature and no interactions with Cuban researchers about the use of baculoviruses for pest management, it seems that there are no large programmes utilising entomopathogenic viruses in Cuba.

Key pests of many annual crops and vegetables worldwide include the genera Heliothis and Helicoverpa, which cause yearly losses in the millions of dollars. An major development in the utilisation of viruses occurred when an NPV of H. zea was developed and registered in the USA in the s. ElcarTM, created by Sandoz, was the first viral insecticide approved for use in cotton in the USA. The HzSNPV has a rather wide host range, infecting several species of the Helicoverpa and Heliothis genera. In Australia, Helicoverpa armigera on cotton has been controlled using a HzSNPV-formulated product known as GemStarTM. Additionally, locally generated isolates of the H. armigera SNPV have been used in China, India, and Australia on cotton, soybean, pigeon pea, maize, and tomato crops.Given that H. zea and H. virescens in the Americas and H. armigera in Africa, Asia, and Australasia both cause significant losses to various crops and vegetables, there is a tremendous potential use for the NPVs of these two pathogens. Over 0 hectares of H. armigera NPVs are utilised yearly in China, including at least HaSNPV producers.

The Spodoptera complex, which includes S. frugiperda, S. exigua, S. littoralis, and S. litura, is another insect genus that has a significant negative economic effect on food production. An indigenous isolate of S. frugiperda NPV was administered to,0 ha/year in Brazil in order to suppress the bug in maize. This programme has been temporarily suspended since no SfNPV products are now accessible to maize farmers due to the challenges and high cost of SfNPV manufacture by the Brazilian Organisation of Agricultural Research, a government research organisation. Currently, S. exigua NPVs have been employed in the USA, Europe, China, and Thailand to control this pest on vegetable crops. These NPVs go by several trade names. Additionally, S. litura's NPV is used in China, India, and Thailand.

The coddling moth, Cydia pomonella, GV, for use in orchards, especially those with apples and pears, may be one of the most significant triumphs in commercial development and application of a baculovirus in Europe. The CpGV has been manufactured and used in a number of nations, including Argentina, Canada, France, Germany, Russia, and Switzerland, under several trade names. The product Madex®, which was first created to aid European organic fruit growers, is currently manufactured for use on more than 0 hectare units every year. Taking into account the use of other CpGV trade names, this might be the most significant viral insecticide now used in the globe in terms of treated area.

CONCLUSION

In conclusion, the use of in vitro baculovirus products has revolutionized the field of biotechnology by providing a safe and efficient method for protein expression in a variety of applications. Baculovirus expression systems have several advantages over traditional methods of protein expression, including high yields, post-translational modifications, and ease of scale-up. In vitro baculovirus products have been used in various fields, including biomedical research, vaccine development, and drug discovery. The ability to produce complex proteins, such as viral antigens and recombinant enzymes, has allowed for the development of novel therapies and diagnostic tools.The use of in vitro baculovirus products

is also beneficial from an environmental standpoint, as it reduces the need for animal-based protein expression systems and eliminates the risk of contamination by human pathogens. However, challenges still remain in the optimization of the baculovirus expression system, including improving the efficiency of gene delivery and reducing the risk of immune responses. Further research is needed to overcome these challenges and to expand the use of in vitro baculovirus products in biotechnology. Overall, in vitro baculovirus products offer a safe, efficient, and versatile method for protein expression, with numerous applications in the fields of biotechnology, medicine, and beyond.

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