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PRINCIPLES OF BIOFILMS

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CHAPTER 1

HISTORICAL BACKGROUND OF BIOFILMS

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ABSTRACT:

Since both Leeuwenhoek and Pasteur described this phenomenon, the observation of aggregated microbes encircled by a self-produced matrix attaching to substrates or located in structures or secretions is as ancient as microbiology. Biofilms have been demonstrated in environmental and technological microbiology to be crucial for contaminants on submerged surfaces, such as ships, 80–90 years ago. The theory of biofilm illnesses and their significance in healthcare is, however, less than 40 years old and started by Jendresen's findings of acquired tooth pellicles and my findings of heaps of *Pseudomonas aeruginosa* tissues in sputum and the lungs tissue from chronically infected cystic fibrosis individuals. In 1985, Costerton introduced the word "biofilm" into medicine. In the decades that followed, it became clear that biofilm infections are common in medicine, and their significance is now widely acknowledged.

KEYWORDS:

Biofilms Composition, Bacterial Biofilms, Bacterial Community, Medical, Robert Koch.

INTRODUCTION

There are both monospecies and poly-species of microbial biofilms, which are described as "a structured consortium of microbial cells surrounded by a self-produced polymer matrix". Biofilms can stick to surfaces, reside in tissue, or be found in fluids, and they can contain components from the host [1].

Long before scientists had the tools to thoroughly investigate them, microbial communities attached to surfaces (biofilms) were observed. In a report to the Royal Society of London in 1684, Anthony van Leeuwenhoek (Figure.1) made the following observation about the massive buildup of microorganisms in dental plaque: "The number of these animalcules in the scurf of a man's teeth are so many that I believe they exceed the number of men in a kingdom."

Midway through the 1800s, Robert Koch invented techniques for producing a solid nutrient medium that could be used to cultivate and separate pure cultures of microorganisms, which marked a significant turning point in the study of microbes. Huge improvements in industry, agriculture, and medicine were the result of this growth. However, because these developments were founded on such a crude understanding of microbial life, many of the solutions' they produced are now being undone. We had no idea that microorganisms would be so much more complicated to study.

H. Heukelekian and A. Heller published "Surfaces enable bacteria to develop in substrates otherwise too dilute for growth" in the Journal of Bacteriology in 1940. Either bacterial slime or colonial growth affixed to surfaces is how development occurs. Many of the basic traits of attached microbial communities were first identified by Claude ZoBell in the 1940s. Numerous papers about microbial films or slime layers were published in the latter half of the 20th century; German researchers occasionally referred to them as "Schmutzdecke."

Figure 1: Anthony van Leeuwenhoek: Diagramed showing the picture of Anthony van Leeuwenhoek (Montana state university)

It became useful to use a specific term to characterize microbial communities because their distinct characteristics from those of planktonic microbes became more obvious. Before "biofilm" was accepted as a word that could be used in publications, researchers used it informally for a while. The term "biofilm" was first used in print in 1975 in the paper "Microbial film development in a trickling filter" by Mack WN, Mack JP, and Ackerson AO in the journal Microbial Ecology. The transmission and scanning electron microscopes were used to visualize the sequence of the biofilm development in the trickling wastewater filter, according to the first line of the abstract. (This one was courtesy of Paul Stoodley. (If you are aware of an earlier publication that contained the term "biofilm," kindly let us know; we will be delighted to correct it.)

Early researchers on biofilms looked at how they affected wastewater filtration, industrial machinery biofouling, and dental plaque (Leeuwenhoek would have been pleased). Biofilms are essentially everywhere because bacteria tend to attach to surfaces. Additionally, biofilm development is linked to microbially influenced corrosion (MIC), product contamination, illnesses from medical devices, and chronic wounds. Additionally, biofilm can have advantageous effects, particularly in polluted soils and water pretreatment systems.

In 1990, recognizing the significance of the microbial activity, as well as the tremendous economic costs associated with microbial communities on surfaces, the US National Science Foundation founded the Center for Biofilm Engineering at Montana State University in Bozeman (though, interestingly, NSF would not initially accept the word “biofilm” in the Center’s name; instead the award funded the “Center for Interfacial Microbial Process Engineering”). Since then, the study of biofilms has proliferated. In order to better comprehend the mysteries of microbial community interactions, new methods, and tools are constantly being developed. There are numerous research facilities studying biofilms in the US, as well as in Denmark, England, Germany, Australia, and Singapore, among other countries.

Communities of microorganisms known as biofilms adhere to surfaces and one another. The slime on stream rocks (and even in hot springs), laundry machines, and even our bodies, like the dental plaque on our teeth, all contain biofilms. Biofilms come in a wide range of compositions and structures (figure.2) Though at the moment the majority of research focuses on single and multi-species bacterial biofilms, they can be made up of a single microbial species or mixed species (such as bacteria and fungi), and these function as an organized community, sharing resources for growth and survival. The creation of biofilms significantly improves microorganism survival by offering structural support and defense against external threats like antimicrobials, grazing predators, and the host's immune system fighting infections.

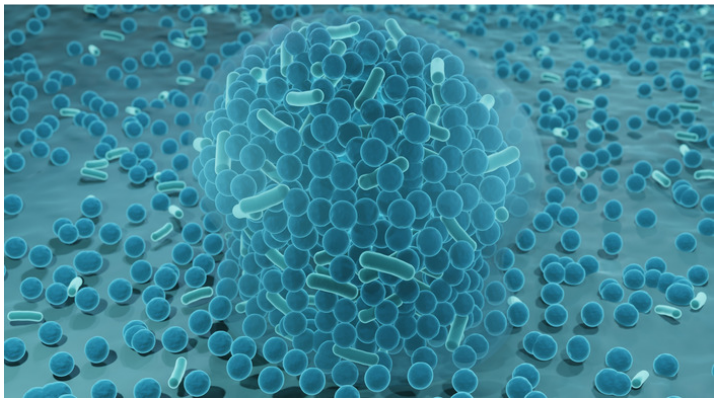

Figure 2: Biofilms: Diagramed the structure of the biofilms (Eurek alert).

To construct a biofilm the investigation of biofilms takes three major directions. The first method involves direct sampling and visualizing the biofilm, whether it is on a pipe, a colonized medical device, the surface of a submerged glass slide or boulder in a creek or pond, or sputum from a person with cystic fibrosis (pwCF). Using a range of stains in conjunction with bright-field microscopy, scanning or transmission electron microscopy (SEM/TEM), or fluorescent microscopy, including confocal scanning laser microscopy, are some of these direct visualization techniques. When coupled with fluorescent in situ hybridization (FISH), these direct visualization techniques offer morphological data as well as significant insight into the bacteria that make up these communities. The second basic strategy creates an in vitro biofilm using flow devices. The Robbins device, numerous other flow devices that can simulate industrial processes, drip reactors, the CDC biofilm reactor, model flow cells, and increasingly, microfluidic devices are some examples.

These tools give one the ability to regulate the bacteria that create the biofilm, tune environmental factors (such as nutrients, pH, oxygen, and temperature), and easily visualize the community over time. The third method, using plastic dishes, enables investigation of the initial stages of biofilm development and, crucially, does so in a high throughput manner suitable for carrying out genetic screens. Each strategy can be used to address specific facets of biofilm biology and, in a way, can be used to operationalize the biofilm. Each strategy also has relative strengths and weaknesses. Because this nidus of infection cannot be cleared by conventional antibiotic treatment, the SEM/TEM of an endocarditis infection, for instance, may be described as being biofilm-based. Another illustration of how the term "biofilm" can apply is when a flow cell-grown community forms spot with a lectin that targets an extracellular polysaccharide. An alternative definition is "bacteria that are attached to a surface in sufficient numbers to be detected macroscopically" in the setting of a microtiter plate assay, indicating robust growth rather than a monolayer of cells. Changes in morphology, physiology, or gene/protein expression can all be defined as discrete

developmental stages that a microbe goes through as it develops from a single cell to a "mature biofilm" using microtiter assays and flow cells). All of these methods are therefore legitimate models for studying biofilms, but none of them fully captures how these communities emerge, their characteristics and functions, and/or their effects on the immediate environment.

LITERATURE SURVEY

Since Leeuwenhoek and Pasteur both characterized the occurrence, the observation of aggregated microbes encircled by a self-produced matrix adhering to surfaces or found in tissues or secretions is not new. In environmental and technological microbiology, biofilms have been demonstrated to be crucial for biofouling on submerged surfaces, such as ships, 80–90 years ago. However, the discovery of masses of *Pseudomonas aeruginosa* cells in sputum and lung tissue from persistently infected cystic fibrosis patients in the early 1970s gave rise to the idea of biofilm infections and their significance in medicine. J. W. Costerton coined the word "biofilm" in medicine in 1985. It was demonstrated that both adhering and non-adhering biofilm infections are common in medicine during the ensuing decades due to the rapid growth in the number of published biofilm papers and techniques for studying biofilms. Guidelines for prophylaxis, diagnosis and treatment have been written, and it is now widely acknowledged how important biofilm infections are from a medical standpoint [2].

Several small perceptions that came together gradually to form the biofilm concept have now been combined and synthesized to create a major "wave" that will take microbiology well into the next century. We can now study bacteria where they reside and go about their daily activities as members of intricate biofilm communities thanks to dozens of novel techniques that have emerged. The identification and management of biofilm populations have become a more important component of corrosion control. The goal of the early biofilm researchers was to identify and quantify bacteria in different ecosystems before describing how they formed functional consortia within highly protected sessile communities using primarily morphological methods. The emergence of device-related and other chronic bacterial infections, as well as the infectious disease community's failure to control these infections or explain why they were refractory, served as the impetus for early medical biofilm microbiology. At the fifth International Society for Microbial Ecology meeting in Osaka, Doug Caldwell's team presented us with the confocal scanning laser microscope, which allows for the examination of living, hydrated specimens on opaque surfaces. Always keep in mind that all techniques for tracking gene expression in biofilms are "average," just like they do in planktonic cultures and that they only reveal whether a gene is up-regulated in some cells, not which ones or where they are located [3].

To investigate the normal course of Staphylococcus epidermidis infection of vascular prosthetic grafts, a mouse model was created. By implanting Dacron prosthetics colonized in vitro with slime-producing *S. epidermidis* to create an adherent bacterial biofilm [1.7 107 colony forming units (CFU)/cm2 graft], graft infections were created in the back subcutaneous tissue of 46 mice. The sterile Dacron prosthetics were implanted in the control animals (n = 16). The comparison animals did not experience graft infection or cutaneous sinus tract grafts. All test animals experienced the development of a biofilm graft infection with usual anatomic (perigraft abscess), microbiological (low bacterial concentration in surface biofilm), and immunologic (normal white blood count) features. In comparison to infected grafts explanted at 2 and 4-6 weeks (1 of 25) and controls (0 of 16), a significantly higher proportion of mice with infected grafts by 8-10 weeks (9 of 21) developed a graft-cutaneous sinus tract. By 8–10 weeks, 2 animals showed no symptoms of graft infection, and 7 grafts could not be recovered with the *S. epidermidis* study strain. Similar to human

infection, bacterial biofilm vascular prostheses infection in mice led to a persistent inflammatory process that oddly manifested as a perigraft abscess or graft-cutaneous sinus tract [4].

In the early days of microbiology, both Leeuwenhoek and Pasteur observed aggregated microorganisms encircled by a self-produced matrix clinging to surfaces or found in tissues or secretions. Biofilms have been demonstrated in environmental and technological microbiology to be crucial for biofouling on submerged surfaces, such as ships, 80–90 years ago. The concept of biofilm infections and their importance in medicine is, however, < 40 years old and was started by Jendresen's observations of acquired dental pellicles and my observations of heaps of *Pseudomonas aeruginosa* cells in sputum and lung tissue from chronically infected cystic fibrosis patients. In 1985, Costerton introduced the word "biofilm" into medicine. The prevalence of biofilm infections in medicine became clear over the ensuing decades, and their significance is now widely acknowledged [5].

CONCLUSION

Research on biofilms has exploded since the late 1990s, which was predominated by speakers from the Center for Biofilm Engineering at Montana State University and labs from England, Denmark, and Germany. Observing researchers from a variety of disciplines, including microbiology, engineering, ecology, physics, chemistry, and more, contribute their knowledge to the study of these intricate and fascinating communities has been both thrilling and enjoyable. In conclusion, the observation of aggregated microorganisms adhering to surfaces or located in tissues or secretions and surrounded by a self-produced matrix is as old as microbiology, but the concept of biofilm infections and their importance in medicine, especially concerning chronic infections, is only 40 years old. It has since become accepted that biofilm infections are frequent and important, but clinical microbiologists have not yet developed methods that are suitable for routine examinations or reports to clinicians of the properties of biofilm-growing microorganisms during daily diagnostic work on samples from patients, and there is only consensus on the treatment of a few biofilm infections. Hopefully, the ESCMID working group on biofilm standards will result in advancements in this field.

REFERENCES:

[1] *Community Structure and Co-operation in Biofilms*. 2000. doi: 10.1017/cbo9780511754814.

[2] N. Høiby, "A short history of microbial biofilms and biofilm infections," *APMIS*. 2017. doi: 10.1111/apm.12686.

[3] J. William Costerton, "A SHORT HISTORY OF THE DEVELOPMENT OF THE BIOFILM CONCEPT," in *Microbial Biofilms*, 2004. doi: 10.1128/9781555817718.ch1.

[4] T. M. Bergamini, R. A. Corpus, K. R. Briitian, J. C. Peyton, and W. G. Cheadle, "The natural history of bacterial biofilm graft infection," *J. Surg. Res.*, 1994, doi: 10.1006/jsre.1994.1062.

[5] N. Høiby, "A personal history of research on microbial biofilms and biofilm infections," *Pathogens and Disease*. 2014. doi: 10.1111/2049-632X.12165.

CHAPTER 2

BIOFILMS FORMED BY THE BACTERIAL COMMUNITY

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ABSTRACT:

Bacteria have the capacity to create biofilms as a universal trait. Multicellular colonies known as biofilms are held together by a self-made extracellular matrix. Biofilms are defined as a community of microorganisms that are attached to a surface, or a group of microorganisms themselves forming microbial aggregates, that are encased within an extracellular matrix (ECM) of polysaccharides, proteins, and glycoproteins, referred to as the extracellular polymeric substance (EPS). Different bacteria use different mechanisms to create biofilms, and these mechanisms frequently rely on the environment and particular strain characteristics. We examine the main characteristics of biofilms and the mechanisms by which extracellular signals cause biofilm formation in this chapter using these bacteria as examples.

KEYWORDS:

Bacterial Biofilms, Extracellular Polymeric, Functional Biofilms, Prokaryotic Cells, Pseudomonas Aeruginosa.

INTRODUCTION

Prokaryotes are believed to have developed biofilms during the early Earth's history as a defense mechanism because the environment was too hostile for them to survive. They can be found as both bacteria and archaea very early in Earth's history, about 3.25 billion years ago. They frequently safeguard prokaryotic cells by maintaining their homeostasis, which promotes the growth of intricate interactions between the cells in the biofilm. Any syntrophic group of microbes in which the cells adhere to one another and frequently to a surface is known as a biofilm. Extracellular polymeric substances (EPSs) make up the slimy extracellular matrix in which these adherent cells eventually become lodged. The extracellular polysaccharides (EPS), which are usually a polymeric mixture of proteins, lipids, and DNA, are produced by the cells that make up the biofilm. They have been metaphorically referred to as "cities for microbes" due to their three-dimensional structure and representation of a community culture for microorganisms.

In natural, industrial, and medical contexts, biofilms can develop on living or non-living surfaces (Figure 1). They could make up a microbiota or be a part of one. In contrast to planktonic cells of the same organism, which are single cells that may float or swim in a liquid medium, microbial cells growing in biofilms are physiologically different from planktonic cells of the same organism. Most animals' teeth can develop biofilms in the shape of dental plaque, which can lead to tooth decay and gum disease. In reaction to a variety of stimuli, including nutritional cues, the exposure of planktonic cells to sub-inhibitory concentrations of antibiotics, and cellular recognition of particular or non-specific attachment sites on a surface, microbes can develop biofilms. When a cell changes to the biofilm mode of growth, it experiences a phenotypic change in behavior that involves the differential regulation of a sizable set of genes.

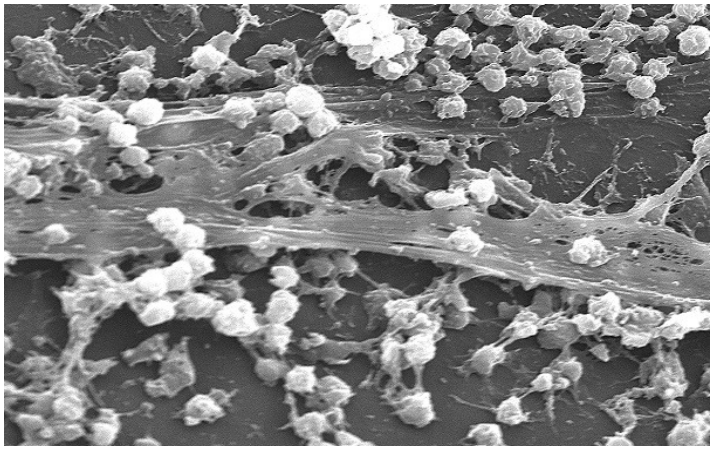

Figure 1: Bacterial biofilms A diagram illustrating how the bacterium biofilms are organized (Wikipedia).

A hydrogel, a complicated polymer that has water content many times its dry weight, is another term for a biofilm. In biofilms, which are more than just bacterial slime layers, the bacteria arrange into a well-organized functional community. A single species of microorganisms or a diverse collection of microorganisms may be present in biofilms, which can adhere to surfaces like a rock or tooth. To support the biofilm's general success, subpopulations of cells within it differentiate to carry out various tasks for motility, matrix production, and sporulation. The bacteria living in biofilms can exchange nutrition and are protected from environmental hazards like desiccation, antibiotics, and the immune system of the host body. Normally, a free-swimming bacterium adheres to a surface, which triggers the formation of a biofilm.

Extracellular DNA (eDNA), polysaccharide intercellular adhesion (PIA), and proteins have been demonstrated to be ECM components in the biofilm of *Staphylococcus aureus* and *S. epidermidis*. Different staphylococcal strains contribute differently to the development of biofilms. While the formation of staphylococcal biofilms depends on PIA production, some strains also produce PIA-independent biofilms. It is also well known that different genotypes have different ECM protein profiles. At least three different alginate exopolysaccharides containing Pel and Psl are produced by *Pseudomonas aeruginosa*, and they each play a role in the growth and structure of biofilms. Mucoid strains create uneven biofilms and overproduce alginate. Both the early biofilm formation and the stability of mature biofilms depend on alginate. Pel and Psl are implicated in the formation of biofilms in non-mucoid strains, which lack the genes necessary for alginate biosynthesis.

Given that it has been demonstrated to attach to Psl directly, the secreted protein CdrA functions as a structural element of the *P. aeruginosa* biofilm matrix. Intercellular communication is facilitated by eDNA, which also aids in maintaining the *P. aeruginosa* biofilm. *P. aeruginosa's* ability to produce biofilms is inhibited by DNase I, which suggests that eDNA is necessary for the initial development of the biofilm. It has recently been demonstrated that during biofilm development, *Haemophilus influenzae* creates an ECM made up of proteins, nucleic acids, and a -glucan. Additionally, it appears that eDNA plays a crucial role in the preservation of biofilms and is a crucial part of the ECM. There are some prerequisites for the beginning of biofilm formation, including the ability of the bacteria to attach to and move on surfaces, sense their cell density, and eventually create a 3-D mesh of cells encased in exo-polysaccharide [3]. Extracellular carbohydrates, signaling molecules, and cell membrane proteins all play significant roles [2] (Figure 1).

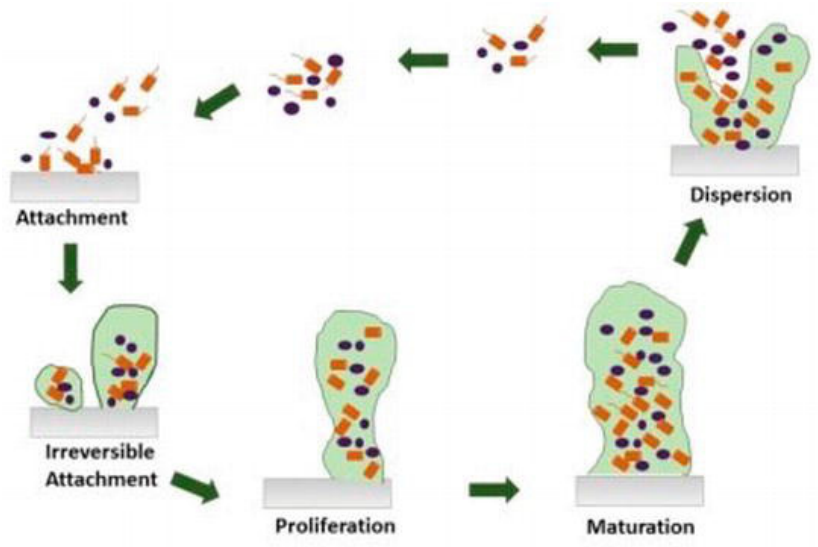

Figure 2: The steps in the creation of bacterial biofilms. Figure illustrating the processes by which bacterium biofilms are formed (intechopen).

Step 1: Attachment: A loose layer of proteins and carbohydrates that combine with minerals in hard water to create a conditioning layer is formed. The bacteria cells are drawn to it and become affixed to the surface. Step 2: Irreversible attachment: As soon as the conditioning layer has developed, an electrical charge begins to build up on the surface, attracting bacteria with the opposite charge and leading to the microbial cells' irreversible attachment. The mild cleanser and sanitizers could readily eliminate microorganisms because the charges are so weak. Step .3 Proliferation: EPS (extracellular polymeric substance), which traps the cells inside a matrix resembling glue, helps bacteria become bonded to surfaces as well as to one another during this period. Step 4: Maturation. The nutrient-rich layer that makes up the biofilm environment supports the rapid development of microorganisms.

A mature biofilm contains complex diffusion channels that transport nutrients, oxygen, and other essential elements for bacterial development while also removing waste materials and dead cells. Step 5: Dispersion Actively growing cells progressively shed their daughter cells during this process of biofilm dispersion (Figure.2). Because biofilm grows as long as new nutrients are kept available, and when they run out of nutrients, they detach from the surface and go back to being planktonic. This procedure most likely takes place to enable bacterial cells to obtain enough nutrients. As *Pseudomonas* fluorescence recolonizes the surface after roughly 5 hours, *Vibrio harveyi* after 2 hours, and *Vibrio parahaemolyticus* after 4 hours, it is also possible that the separation process is species-specific. Intimate links between EPS's functional part and the biofilms' emergent properties have been discovered over time. Dental biofilm studies are a great source of data for understanding the make-up and physiological functions of the EPS matrix.

we use the term ‘matrixome’, adapted from ‘matrisome’ used traditionally in the field of eukaryotic cell biology, to define the entire inventory of currently known biomolecules, and their molecular, structural, and functional diversity, associated with biofilm assembly and its physicochemical and virulence attributes. The variety of microorganisms, the shear stress in the area, the abundance of nutrients and substrates, and the host environment can all have a significant impact on the composition and structure of EPS. Between monospecies and multispecies microbial communities, EPS synthesis and spatial structure vary. A wide variety of proteins have so far been discovered (Figure.4). These can be divided into two categories: (i) cell surface-associated and (ii) extracellularly released. (Figure. 3). Examples include the

functional amyloids, type IV pili, and flagella that are attached to cells and regulate bacterial adhesion, mechanical stability, and autoimmune reactions.

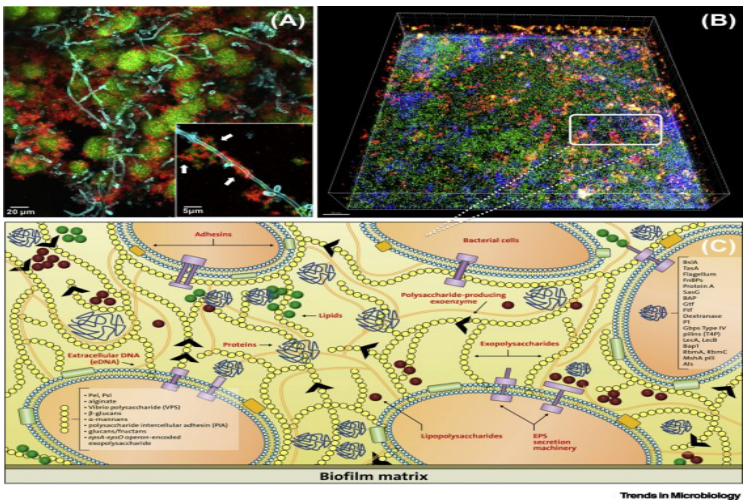

Figure 3: Shows the bacterial biofilms' composition. Diagrams illustrating the various biofilm compositions (cell press).

On the other hand, secreted bacterial proteins, eDNA, and eRNA that are released extracellularly help the matrix to operate and form scaffolding. Although we concentrate on EPS generated by microorganisms, the matrix come should also include biomolecules obtained from the host or environment. It has been discovered that host proteins and glycoproteins, such as the salivary proteins, help to build the matrix framework and facilitate microbial attachment while acting as a source of nutrients for the microbes. The morphological and functional characteristics of the biofilm, which can be broadly classified into physical and chemical properties, have been shown to depend critically on the EPS components. The formation of biofilms is a crucial adaptation and survival tactic frequently used by bacteria. The EPS shields bacteria in the biofilm from harmful environmental variables and immune responses.

Class	Microorganism[a]	Name	Location	Function
Polysaccharides	*Bacillus subtilis*	*epsA-epsO* operon-encoded exopolysaccharide	Extracellular	Adhesion, scaffolding, stability
		γ-PGA (poly-γ-glutamate)	Extracellular	Adhesion, scaffolding, sorption, nutrient
	Staphylococcus aureus	Polysaccharide intercellular adhesin (PIA) or poly-β (1-6)-*N*-acetylglucosamine (PNAG)	Extracellular	Adhesion, cohesion, scaffolding, stability, protection against antibiotics
	Streptococcus mutans	Glucans/fructans	Extracellular/cell-associated	Adhesion, cohesion, scaffolding, stability, cell-to-cell binding, acidic microenvironment, protection against antimicrobials, nutrient
	Pseudomonas aeruginosa	Psl	Extracellular/cell-associated	Adhesion, scaffolding, stability, protection against immune response, cell-to-cell binding
		Pel	Extracellular/cell-associated	Adhesion, scaffolding, stability, cell-to-cell binding, protection against antibiotics
		Alginate	Extracellular	Adhesion, scaffolding, water/nutrient retention, protection against harsh environments/immune response/antimicrobials, stability
	Vibrio cholerae	*Vibrio* polysaccharide (VPS)	Extracellular/cell-associated	Adhesion, cohesion, scaffolding, stability
	Candida albicans	α-mannans	Extracellular Cell wall	Forming mannan-glucan complex (MGCx), scaffolding, protection, antifungal resistance, bacterial–fungal interaction
		β-glucans	Extracellular Cell wall	Forming mannan-glucan complex (MGCx), scaffolding, protection, antifungal resistance, bacterial–fungal interaction
Proteins	*Bacillus subtilis*	Biofilm surface layer protein (BslA)	Extracellular	Surface hydrophobicity, protection
		Translocation-dependent antimicrobial spore component (TasA)/TasA anchoring and assembly protein (TapA)	Extracellular Cell wall	Scaffolding, cell-to-cell binding
		Flagellum	Cell-associated	Adhesion, motility, mechanosensing
	Staphylococcus aureus	Fibronectin-binding proteins (FnBPs)	Cell-associated/extracellular	Adhesion, cell-to-cell binding
		Staphylococcal Protein A (SpA)	Cell-associated/extracellular	Adhesion, cell-to-cell binding, immune evasion
		S. aureus surface protein G (SasG)	Cell-associated/extracellular	Adhesion, cell-to-cell binding
				Adhesion, cell-to-cell binding, scaffolding

Figure 4: Bacterial biofilms: Diagram Showing the composition of the bacterial biofilms.

LITERATURE SURVEY

In nature, communities of bacteria create surface-attached biofilms. Bacterial cells within biofilms are resistant to sanitizers and antimicrobials, in contrast to free-living cells. Cells physiologically adjust while forming biofilms to withstand the otherwise fatal effects of various environmental stress conditions. The creation and encapsulation of cells in extracellular polymeric materials are crucial to this growth. Numerous issues with food preparation, such as decreased heat-cold transfer, clogged water pipes, food spoilage, and the potential for consumer infections, can be brought on by biofilm bacteria. A combination of bacterial genetics, systems biology, materials and mechanic engineering, and chemical biology has been used in recent biofilm studies to develop potential control methods [2].

It is now widely accepted that the majority of bacterial life in nature, as opposed to isolated planktonic cells, is found in surface-bound colonies known as biofilms. More than 80% of chronic inflammatory and infectious illnesses are attributed to biofilms. Numerous chronic diseases can be rethought of as biofilm diseases thanks to the biofilm paradigm. Even between different species, the biofilm bacterial community employs secreted pheromones (such as quorum sensing molecules) and other molecules for cell-cell signaling. The bacterial community gains many advantages from these coordinated actions that take place during the formation of biofilms. Biofilms offer safety against host defenses and resistance to many antimicrobials. The proportion of persister cells within the biofilm appears to have increased, which could be one explanation for the greater resistance to environmental stresses and antibacterials seen in biofilm cells [3].

Bacterial biofilm development exhibits several striking similarities to the formation of higher organisms, including the early social behavior of undifferentiated cells as well as cell death and differentiation in the mature biofilm. Recent developments in the area offer fresh insight into cell differentiation and death processes during the formation of bacterial biofilms and suggest that biofilms exhibit an unexpected degree of multicellularity[4].

According to Vitruvius' essay "On Architecture," architecture is merely an imitation of nature. Here, we talk about what occurs when nature is used in architecture. We outline recent advances in the study of biofilm structure and suggest fusing contemporary architecture with synthetic microbes to create methods for sustainable building. A role for calcium carbonate precipitation in the maturation and assembly of bacterial communities with complex structures was recently disclosed by the Kolodkin-Gal laboratory and others. Importantly, they showed that various organic materials secreted by the microbes shape the calcium carbonate crystals that they produce. This serves as a proof-of-concept for the possible application of bacteria in the design of rigid building materials and the modification of crystal morphology and function. In this research, we examine how these recent discoveries might alter the conventional wisdom regarding architecture and buildings. We think that carbon dioxide can be absorbed while also constructing structures using biofilm communities that have been enhanced by synthetic circuits [5].

Microbial communities that are affixed to surfaces and enclosed in an extracellular matrix also made by microbes are known as biofilms. They stand in for the predominant microbial living form. There are biofilms everywhere, and they can grow on almost any surface, both natural and artificial. Additionally, biofilms are found everywhere in both healthy and unhealthy human systems. Numerous pathogens could create biofilms, and it is obvious that this is one of the primary means by which bacteria survive in the human body's various environments. The biofilm culture aids bacterial survival and persistence in the environment almost always. In this overview, the basic biology of microbial biofilms is covered, along

with how biofilms affect the pathogenesis of human infections. In this review, the various mechanisms that contribute to pathogenic microorganisms' decreased antimicrobial susceptibility are covered in depth. Also provided are potential strategies that might be investigated in the hunt for fresh anti-biofilm tactics to get rid of medically important biofilms [6].

Even seemingly unrelated occurrences like the fouling of ships and the development of chronic lung infections by *Pseudomonas aeruginosa*in cystic fibrosis patients are affected by bacterial biofilms because these bacteria are more resilient to antimicrobial therapy and human defense mechanisms. It was widely believed that these stark differences would result in significant variations in bacterial gene expression1. But a recent issue of Nature contains a paper by Marvin Whiteley and coworkers that offers a rather unexpected discovery. A *P. aeruginosa* gene expression analysis reveals that, of the 5570 genes queried by the genomic microarray4, only a relatively small proportion (34 genes) are up- or down-regulated during biofilm development. This stands in stark contrast to the alteration in gene expression caused by other circumstances, such as a lack of Mg2+ in growth media (my private observations) [7].

Biofilms have historically been seen as harmful or troublesome. Biofilms, on the other hand, have advantageous qualities like self-regeneration, sustainability, scalability, and tunability, making them candidates for a variety of uses.

Wild-type or metabolically engineered strains are frequently the foundation for traditional biofilm uses like corrosion protection, bioleaching, microbial fuel cells, and environmental remediation. We also discuss design approaches for numerous creative uses of living functional biofilms in this study. Living functional biofilms have been created by researchers using a variety of techniques, including the merging of signaling pathways, metabolic pathway engineering, and modification of extracellular polymeric substances. In the literature, it has been shown that functional biofilms can be used for a variety of purposes, such as catalysis, electric conduction, bioremediation, and medical treatment. Genetic editing, metal ion curing, synthetic gene circuits, and other techniques can be used to modify the mechanical characteristics of biofilms. Making living, functional biofilms with particular structures have also advanced significantly thanks to the development of 3D printing using bio-inks. Future real large-scale applications of biofilms will result from the fusion of synthetic biology with methods from other disciplines.

CONCLUSION

A complex self-produced matrix of polysaccharides, extracellular DNAs, and proteins surrounds bacteria that have adhered to surfaces to create a biofilm. The development of biofilms is a complicated, regulated process that includes ongoing steps and intricate mechanisms that are controlled by chemical, physical, and biological processes. Biofilms enable the infection to linger, prevent the absorption of antimicrobials, and encourage drug resistance, making it challenging to treat bacterial infections. So far as global public health is concerned, bacterial biofilms are an emerging issue that the general public, medical experts, and the scientific community are all very concerned about. Since large doses of antibiotics are needed to completely eradicate biofilms, current conventional therapies are insufficient for the safe and effective treatment of biofilms. As a result, biofilm treatment requires novel therapeutical approaches. The specifics of bacterial biofilms and how they develop were covered in this chapter.

REFERENCES:

[1] L. Karygianni, Z. Ren, H. Koo, and T. Thurnheer, "Biofilm Matrixome: Extracellular Components in Structured Microbial Communities," *Trends in Microbiology*. 2020. doi: 10.1016/j.tim.2020.03.016.

[2] S. Y. E. Tan, S. C. Chew, S. Y. Y. Tan, M. Givskov, and L. Yang, "Emerging frontiers in detection and control of bacterial biofilms," *Current Opinion in Biotechnology*. 2014. doi: 10.1016/j.copbio.2013.08.002.

[3] M. Jamal *et al.*, "Bacterial biofilm and associated infections," *Journal of the Chinese Medical Association*. 2018. doi: 10.1016/j.jcma.2017.07.012.

[4] J. S. Webb, M. Givskov, and S. Kjelleberg, "Bacterial biofilms: Prokaryotic adventures in multicellularity," *Current Opinion in Microbiology*. 2003. doi: 10.1016/j.mib.2003.10.014.

[5] M. Dade-Robertson, A. Keren-Paz, M. Zhang, and I. Kolodkin-Gal, "Architects of nature: growing buildings with bacterial biofilms," *Microbial Biotechnology*. 2017. doi: 10.1111/1751-7915.12833.

[6] D. Dufour, V. Leung, and C. M. Lévesque, "Bacterial biofilm: structure, function, and antimicrobial resistance," *Endod. Top.*, 2010, doi: 10.1111/j.1601-1546.2012.00277.x.

[7] R. E. W. Hancock, "A brief on bacterial biofilms," *Nature Genetics*. 2001. doi: 10.1038/ng1201-360.

CHAPTER 3

MYCOTIC BIOFILM OCCURRENCE AND THE DEVELOPMENT IN THE NATURAL SYSTEM

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ABSTRACT:

Communities of adherent cells encased in an extracellular substance make up fungal biofilms. These biofilms are frequently discovered during infections brought on by several different fungal diseases. Due to their resilience to antifungals and host defenses, biofilm infections can be very challenging to treat clinically. Fungal biofilms are a growing health issue that is linked to high mortality rates. The most notorious of all fungi that produce biofilms is *Candida albicans*. However, it has been demonstrated that non-Candida species, including filamentous molds like *Aspergillus fumigatus* and yeasts like *Cryptococcus neoformans*, are responsible for biofilm-associated illnesses. Adhesion, colonization, maturation, and dispersal are some of the different developmental stages of fungal biofilms that are controlled by intricate molecular processes. For individuals with fungus biofilms, resistance to antifungal therapy continues to pose the biggest risk.

KEYWORDS:

Biofilms Infection, Biofilms Production, Candida Species Extracellular Matrix, Fungal Biofilms.

INTRODUCTION

Any member of the eukaryotic group of creatures, which also includes the more well-known mushrooms and microorganisms like yeast and mold, is referred to as a fungus (PL: fungi or funguses). Separate from the other eukaryotic kingdoms, which according to one conventional classification include Plantae, Animalia, Protozoa, and Chromista, these organisms are categorized as a kingdom. The fungus kingdom includes a huge variety of taxa with different ecologies, life cycle tactics, and morphologies, spanning from massive mushrooms to unicellular aquatic chytrids. The real biodiversity of the kingdom of fungi, which has been estimated to contain between 2.2 million and 3.8 million species, is, however, little understood.

Only about 148,000 of these have been described, and more than 8,000 of these species are known to be harmful to plants, while at least 300 are potentially pathogenic to people. Since the groundbreaking taxonomical works of Carl Linnaeus, Christiaan Hendrik Persoon, and Elias Magnus Fries in the 18th and 19th centuries, fungi have been grouped based on their appearance or physiology (e.g., traits like spore color or microscopic features). The incorporation of DNA analysis into taxonomy has become possible thanks to developments in molecular genetics, which has occasionally challenged the historical classifications based on morphology and other characteristics. The classification within the fungi kingdom, which is broken down into one subkingdom, seven phyla, and ten subphyla, has been changed as a result of phylogenetic studies released in the first decade of the twenty-first century.

One of the main forms of microbial proliferation, biofilms are essential for the emergence of clinical infection. A wide variety of microbial diseases in the human host are caused by them.

Numerous crucial fungi for medicine, such as *Candida Aspergillus, Cryptococcus, Trichosporon, Coccidioides,* and *Pneumocystis*, create biofilms (Figure.1) in this study, we focus on characteristics that are shared by fungus biofilms and identify potential conserved genes and pathways.

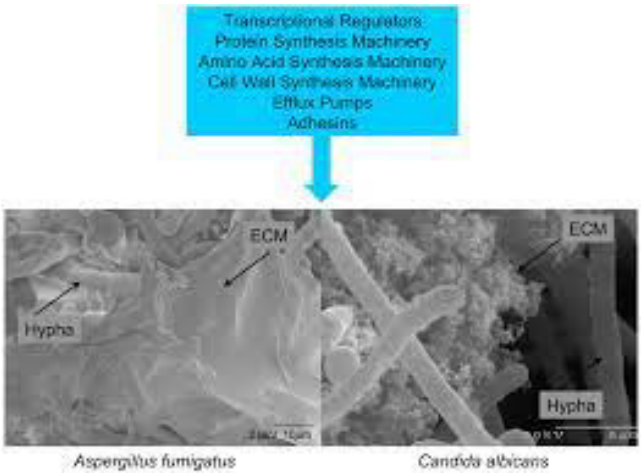

Figure 1: Fungal biofilms: Diagrammed showing the organization of the fungal biofilms (PLOS).

Antifungal medication resistance is higher in biofilm cell communities than in planktonic cell communities. Extracellular matrix (ECM), metabolic heterogeneity inherent to biofilms, and biofilm-associated up-regulation of efflux pump genes are some of the contributing variables. The real resistance increase differs depending on the drug and the species. Fluconazole, amphotericin B, nystatin, voriconazole, and other antifungal medications have little effect on the biofilms of Candida albicans and *Candida parapsilosis*. Itraconazole and, to a lesser degree, caspofungin is relatively ineffective against *Aspergillus fumigatus* biofilms. Fluconazole and voriconazole do not affect cryptococcal biofilms, and *Trichosporon asahii* biofilms exhibit increased resilience to amphotericin B, caspofungin, voriconazole, and fluconazole. Anti-Pneumocystis carinii biofilm treatments with azide and amphotericin B are ineffective. In *C. albicans* and *A. fumigatus*, biofilm-associated resistance mechanisms have been identified, and these include the generation of persister cells and the binding of drugs to the ECM. Only a small portion of the population consists of persister cells, which likely indicates the population's metabolic heterogeneity. These processes might also apply to other fungi [1].

The zinc transporters Zrt1 and Zrt2 allow Zap1 to regulate the levels of zinc in cells, which is necessary for *C. albicans* cell activity. For cells to remain viable, the quantity of zinc must be controlled, and cells become toxic when zinc levels rise too high. The zinc ions are transported by the Zrt1 with a high affinity while the zinc ions are transported by the Zrt2 with a low affinity.

Complex surface-associated cell populations known as biofilms, which are embedded in an ECM, have different phenotypes from their planktonic cell peers. Contributing variables include nutrients, quorum-sensing molecules, and surface contact. Yeast-form and hyphal cells make up the majority of *C. albicans* biofilms, and both are necessary for biofilm development. Adherence to a substrate (either abiotic or mucosal surface), yeast cell proliferation over the surface, and stimulation of hyphal formation are all steps in the formation process. ECM builds up as the biofilm gets older and appears to help with structure. Numerous abiotic and biotic substrates produce *C. albicans* biofilms. A mix of

biotic mucosal (the host) and abiotic surface (the denture) biofilm formation occurs in denture stomatitis. Other Candida species, such as *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, generate biofilms that contain ECM but no true hyphae.

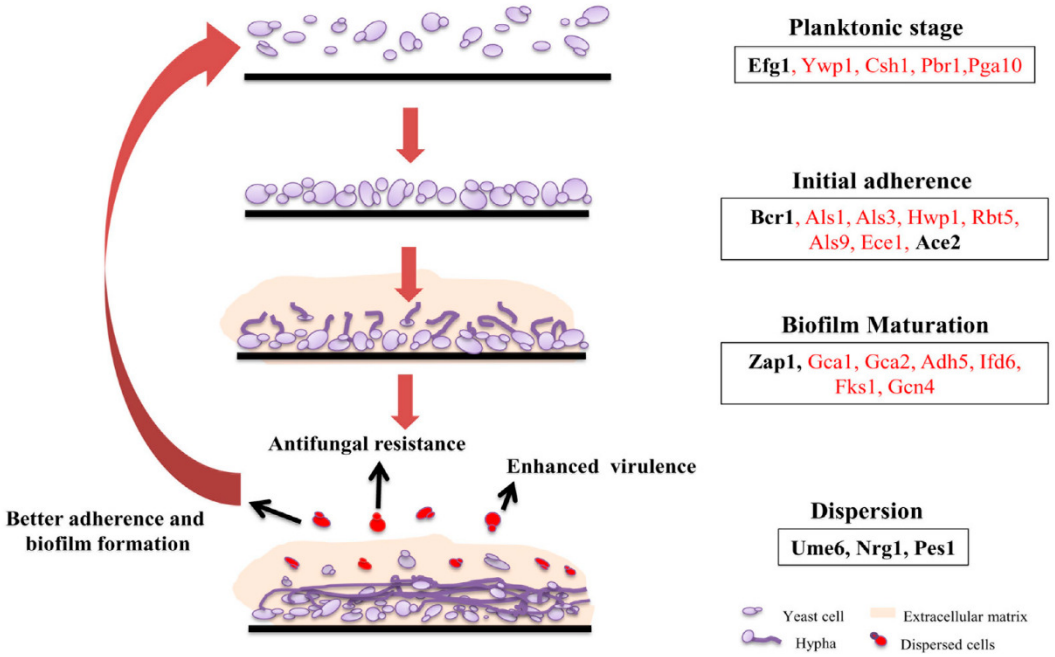

Figure 2: Biofilms formation: Diagramed showing the steps involved in the fungal biofilms formation (MDPI).

Both biotic and abiotic substrates are capable of developing Aspergillus biofilms. Conidia are the early colonizing cells that stick to the substrate. As the biofilm ages, mycelia (the hyphal form) grows. Both in vitro and in vivo observations of the ECM that holds the biofilm together have been made. The two types of *A. fumigatus* biofilm infection have distinct hyphal arrangements: aspergillosis infections have individually separated hyphae, while aspergilloma infections have an intertwined ball of hyphae. Hyphae of *C. albicans* and *A. fumigatus* have the ability to penetrate biotic surfaces and create pores or channels.

Similar to *Coccidioides immitis*, the newly discovered fungal pathogen *T. asahii* creates biofilms from yeast and hyphal cells embedded in matrix. On a variety of abiotic substrates, *C. neoformans* produces biofilms made of yeast cells, and the ECM is made of shed capsular polysaccharides. Although *C. neoformans* produces hyphae during breeding, no hyphae have ever been seen in *C. neoformans* biofilms up until this point. The biofilms produced by Pneumocystis species do not contain any hyphal structures. As a result, hyphal formation is not always present in fungus biofilms.

Opportunistic pathogenic yeast Candida albicans is a frequent constituent of the flora in the human intestine. The human body is not the only place it can thrive. In between 40% and 60% of healthy adults, it is found in the mouth and gastrointestinal system. It is typically a commensal bacterium, but in a variety of immunocompromised patients, it can become pathogenic. As a consequence of an overgrowth of the fungus, it is one of the few species of the genus Candida that can cause the infection of candidiasis in humans. Candidiasis, for instance, is frequently seen in HIV-positive individuals. The most prevalent fungus found in biofilms that have developed on human tissue or (permanently) implanted medical equipment is *C. albicans*. Together, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* account for 50–90% of all human instances of candidiasis. Patients with *C. albicans*-related systemic

candidiasis have a mortality incidence of 40%. Invasive candidiasis acquired in a hospital is thought to result in 2,800 to 11,200 fatalities annually in the US. Nevertheless, given recent studies showing that *C. albicans* can cross the blood-brain barrier in mice, these numbers might not accurately represent the full extent of the harm that this organism causes.

C. albicans is frequently utilized as a fungus pathogen model organism. Because it can develop as both yeast and filamentous cells, it is commonly referred to as a dimorphic fungus. It does, however, come in a variety of morphological phenotypes, such as opaque, GUT, and pseudohyphal varieties. For a very long period, *C. albicans* was thought to be an obligate diploid organism lacking a haploid stage. But this is not the situation. *C. albicans* can also live in a tetraploid stage in addition to a haploid stage. The latter is created when mating pairs of opaque, diploid *C. albicans* cells occur. Up to 70% of the protein-coding genes in the haploid genome, which has a size of about 29 Mb, remain uncharacterized. It is simple to grow *C. albicans* in the lab, and it can be examined both in vivo and in vitro. Different research can be conducted depending on the media because it affects the morphology of Candida albicans. CHROMagar Candida is a unique kind of medium that can be used to distinguish between various Candida species.

Forming a biofilm, stages: *C. albicans* forms its biofilm in four stages. The yeast-form cells first adhere to the substrate in the initial adhesion phase. The second stage is referred to as the Intermediate stage, during which the cells multiply to create microcolonies and germ tubes develop to produce hyphae. The biofilm biomass grows, the extracellular matrix builds up, and drug resistance rises during the maturation phase. The yeast-form cells are released to colonize the surrounding environment during the final stage of biofilm development. Increased virulence and drug tolerance are two new traits of yeast cells that have been liberated from a biofilm (Figure.2).

A transcription factor called Zap1, also referred to as Csr1 and Sur1 (zinc-responsive activator protein), is necessary for the development of hyphae in *C. albicans* biofilms. Zap1 regulates the zinc transporters, zinc-regulated genes, and the balance of yeast and hyphal cells in *C. albicans* biofilms. The mechanisms involved in *C. albicans* biofilm formation and the regulatory circuits that are essential to *C. albicans* biofilm development are summarized in this. We look into the fungal biofilms formation in the environment.

LITERATURE SURVEY

Complex interactions between microorganisms are crucial to the pathogenesis of infections. These interactions can take many different forms, from ferocious competition for resources and niches to highly developed cooperative mechanisms between various species that promote their mutual development. Studies on polymicrobial biofilms in various disease models have replaced monomicrobial biofilm studies due to a growing understanding of these interactions and a wish to understand the mechanisms governing them. In this paper, we give a summary of the biofilm models that have been used to investigate a few specific polymicrobial infections and we emphasize the influence that these biofilms' interactions between microbes have on the development of disease. The difficulties in studying polymicrobial biofilms are discussed, as well as notable new developments in the creation of infection models linked to polymicrobial biofilms[2].

A significant amount of interspecies interactions take place in polymicrobial biofilms, frequently to the host's disadvantage. Polymicrobial biofilms, which frequently display greater resistance to antimicrobial treatment, are blamed for many chronic infections. Nevertheless, despite the seriousness of such illnesses, research into polymicrobial diseases is still in its infancy. Therefore, there is still much work to be done to advance knowledge of

new ideas in the formation of biofilms, such as interspecies communication and host immune response to microbial biofilms. Designing efficient therapeutic approaches to thwart microbial colonization and stop the emergence of polymicrobial diseases is the main challenge. Therefore, future research should concentrate on developing animal model systems to investigate infections and polymicrobial biofilms that are produced in vivo. This review highlights the difficulties and cutting-edge strategies being pursued to fight polymicrobial biofilms and infections while summarizing our scant understanding of the nature of these complex communities and their involvement in disease[3].

A typical fungus found in the human microbiome is Candida albicans. In healthy people, it is typically a harmless commensal, but several factors can cause it to overgrow and result in a variety of complications within the host, from localized superficial infections to systemic life-threatening disseminated candidiasis. The capacity of *C. albicans* to create biofilms, a densely packed community of cells that can grow on both abiotic and biotic substrates, including mucosal surfaces and implanted medical devices, is a key component of its virulence. Biofilm-associated infections are a significant clinical issue because these biofilms are very challenging to eradicate, resistant to traditional antifungal therapy, and associated with high morbidity and mortality rates. In this paper, we examine the current understanding of the processes involved in the formation and development of *C. albicans* biofilms, including the crucial processes of adhesion, the production of extracellular matrix, and the transcriptional network that controls biofilm development. We also look at the benefits of living in biofilms and investigate how different microbial species interact with one another to create multispecies biofilms[4].

Pathogenic fungi have a virulence trait known as biofilm formation. Both yeasts and filamentous fungi are capable of attaching to biotic and abiotic surfaces and growing into highly organized colonies that are tolerant of environmental factors and antimicrobial agents. In recent years, the development of biofilms has been linked to novel fungi genera. However, from a morphological and biochemical standpoint, Candida biofilms continue to be the subject of most research. There are differences between the biofilms produced by yeast and filamentous fungi, and research on polymicrobial communities is becoming more and more essential. The extracellular matrix, which covers and shields biofilm cells from their surroundings, is a crucial component of resilience. Furthermore, quorum-sensing molecules that regulate biological activities and behaviors as well as fungal resistance and pathogenicity are secreted by microbes as a means of achieving cell-cell communication. Several in vitro techniques have been developed to study fungal biofilms, from colorimetric methods to omics approaches that aim to identify new therapeutic strategies by developing new compounds to combat these microbial communities as well as new diagnostic tools to identify these complex formations in vivo. Recent developments concerning pathogenic fungus biofilms are discussed in this review[5].

The most common human fungus, *Candida albicans*, can infect both immunocompetent and immunocompromised people and can live in a variety of host habitats. Additionally, *C. albicans* easily creates biofilms on mucosal tissues, indwelling medical devices, and other surfaces. These biofilms act as an infectious reservoir that is challenging to get rid of and can cause fatal systemic infections. The environment in which biofilm development takes place is complicated and includes both host factors and other human microbes. Polymicrobial interactions are likely to control the biofilm's cellular and biochemical composition as well as therapeutically important outcomes like virulence and host and drug tolerance. In this paper, we discuss the pathogenesis of *C. albicans* infections in the setting of in vivo polymicrobial biofilms[6].

Over ten years ago, the biofilm infection paradigm was first put forth. We now know a lot more about biofilms, typically polymicrobial communities that are frequently linked to chronic infection, thanks to recent scientific advancements. Bacteria using a biofilm strategy have numerous methods for promoting diversity, as shown by metagenomics. By including multiple bacterial and/or fungal species in a single community, biofilms obtain numerous advantages, such as passive resistance, metabolic cooperation, byproduct influence, quorum sensing systems, an enlarged gene pool with more efficient DNA sharing, and many other synergies, which give them a competitive advantage. Regular clinical cultures are inadequate for assessing illnesses caused by multiple microbes. In clinical infections, DNA techniques employing PCR, PCR/mass spectroscopy, and sequencing have shown their capacity to identify microorganisms and quantify their contribution to biofilms. Clinical outcomes are being quickly improved by a more reliable model of biofilm infection and more precise diagnosis[7].

Polymicrobial biofilm diseases are playing a bigger part in medicine. We now know more about how both beneficial and harmful microbial interactions affect disease outcomes thanks to an increase in microbiome research and deep sequencing. This is especially important in the oral cavity, a complex and varied ecology where both bacteria and yeasts live in communities known as biofilms and coexist in a variety of niches. Though rarely both together, studies within this environment tend to be the topic of in-depth independent investigation in the context of either polymicrobial bacterial communities or yeast biofilms. But they don't conflict with one another. Therefore, this review aims to explore the influence of candidal populations on the composition of these complex aggregates and biofilm communities, to investigate their mechanistic interactions to understand how these impact clinical outcomes, and determine whether we can translate how this knowledge can be used to improve patient management[8].

CONCLUSION

Because they are one of the numerous creatures that break down organic matter, fungi have a significant impact on the biosphere. A dearth of fungi could cause the ecosystem's cycle to be upset. Decomposition, nutrient cycling, symbiosis, and a food supply are all facilitated by fungi. Numerous fungi have the ability to develop biofilms. The formation of biofilm on implanted devices is a key contributor to recurrent infection, making this growth form significant for the biology of infection. Additionally, biofilms are only weakly drug-susceptible, which makes treating device-associated infections very challenging.

Fungal pathogens called Candida species are well-known for their capacity to attack humans with superficial and systemic infections. The evolution of pathogenicity and multidrug resistance characteristics in these pathogens allows them to survive inside the host, frequently failing therapeutic approaches. The ability of Candida species to create biofilms, which shields them from external elements like the host immune system's defenses and antifungal medications, is one distinctive trait of their pathogenicity. This review emphasizes the differences between the four species and concentrates on the current threats and difficulties associated with dealing with the biofilms produced by *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*. The ability of each species to create extracellular polymeric substances (EPS) and exhibit dimorphic growth, as well as the substratum of the biofilm, the accessibility of carbon sources, and other factors, all affect the biofilm's characteristics. Additionally, pathogenic yeasts of the Candida genus exhibit a high degree of intricacy and diversity in the transcriptional regulation of processes like adhesion, biofilm formation, filamentation, and EPS production. The antifungal resistance that is usually present in Candida biofilm cells, potentiated by EPS, which acts as a barrier to drug

diffusion, and by the overexpression of drug resistance transporters, is affected by these differences, as well as the persistence of colonization and infections. Another crucial aspect to take into account when addressing this issue is the capacity of in vivo Candida biofilms to engage with various species. The most effective approaches to prevent the formation of biofilms are presently being developed or are already in use, despite numerous obstacles.

REFERENCES:

[1] S. Fanning and A. P. Mitchell, "Fungal biofilms.," *PLoS Pathog.*, 2012, doi: 10.1371/journal.ppat.1002585.

[2] R. A. Gabrilska and K. P. Rumbaugh, "Biofilm models of polymicrobial infection," *Future Microbiology*. 2015. doi: 10.2217/fmb.15.109.

[3] M. A. Jabra-Rizk, "Pathogenesis of Polymicrobial Biofilms," *Open Mycol. J.*, 2011, doi: 10.2174/1874437001105010039.

[4] N. O. Ponde, L. Lortal, G. Ramage, J. R. Naglik, and J. P. Richardson, "Candida albicans biofilms and polymicrobial interactions," *Critical Reviews in Microbiology*. 2021. doi: 10.1080/1040841X.2020.1843400.

[5] C. B. Costa-Orlandi *et al.*, "Fungal biofilms and polymicrobial diseases," *Journal of Fungi*. 2017. doi: 10.3390/jof3020022.

[6] M. M. Harriott and M. C. Noverr, "Importance of Candida-bacterial polymicrobial biofilms in disease," *Trends in Microbiology*. 2011. doi: 10.1016/j.tim.2011.07.004.

[7] R. Wolcott, J. W. Costerton, D. Raoult, and S. J. Cutler, "The polymicrobial nature of biofilm infection," *Clinical Microbiology and Infection*. 2013. doi: 10.1111/j.1469-0691.2012.04001.x.

[8] L. E. O'Donnell *et al.*, "Polymicrobial Candida biofilms: friends and foe in the oral cavity," *FEMS yeast research*. 2015. doi: 10.1093/femsyr/fov077.

CHAPTER 4

A BRIEF OVERVIEW OF BIOFILMS IN THE MEDICINE

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ABSTRACT:

A little substance called microbial biofilm has a big impact on people's health. It is made up of bacterial colonies that are shielded from environmental stress, stress caused by shear, chemicals, antimicrobial chemicals, and the immune system of the host by an external polymeric matrix. Biotechnology and Bioengineering studies on microbial biofilms that are created naturally and artificially in aquatic and subterranean ecosystems, waste-gas treatment systems, marine vessels and structures, and industrial bioprocesses for more than 20 years. Engineered biofilms are heterogeneous reaction systems that, in comparison to suspended culture systems, can improve reactor productivity, and system stability, and provide intrinsic cell: product separation. Unwanted biofilms can significantly increase fluid frictional resistances, result in unacceptably low heat transmission rates, contaminate products, hasten corrosion, and worsen material deterioration. The present issues surrounding medical biofilms, current theories on how biofilms form, persist, and interact with the host immune system, and emerging technologies for managing medical biofilms are all covered in this chapter.

KEYWORDS:

Antibiotic Resistance, Biofilms Formation, Human Health, Microbial Biofilms, Medical Device.

INTRODUCTION

Clusters of one or more living microorganisms, such as bacteria, fungi, and viruses that are adhered to a surface and embedded in a self-produced matrix created for the survival of organisms are known as biofilms. Biofilms have a major impact on human health and medicine from a medical standpoint. A major contributor to the contamination of medical equipment and the development of microbial and chronic illnesses in the body is bacterial biofilm. Since they cause severe infections and have characteristics that make them resistant to antimicrobial drugs, biofilms are the cause of several human disorders. Biofilm infections are particularly challenging to treat because the microorganisms that live there are less likely to be impacted by medicines and disinfectants.

Biofilms are frequently a cause for worry in the medical field because of their propensity to develop on implants and their resistance to antibiotics. As a result, biofilms have the potential to result in severe illness and the failure of medical operations and therapies. This is primarily caused by EPS and physiological changes in the bacteria living in biofilms, as these biofilms contain a variety of proteins that are not present in planktonic or free-living cells. Since bacteria that reside in biofilms are frequently resistant to the immune system, antibiotics, and other therapies, biofilm infections are frequently long-lasting. The antibiotic-resistance traits of the biofilm are a serious issue with important repercussions. The persister cells in the biofilm frequently lead to antibiotic resistance. These cells give up propagation to live in the

presence of lethal elements. That is, when antimicrobial treatments are applied, the cells transform into a condition in which they do not divide.

Over 75% of all illnesses are related to and impacted by microbial biofilms. The main four ways that biofilms have an impact are by promoting the development of antimicrobial drug resistance, causing chronic infections, altering the host immune response, and contaminating medical equipment. The biofilm lifestyle also helps microorganisms survive in challenging environmental circumstances.

Single (monomicrobial) or multiple (polymicrobial) species of organisms can coexist to create biofilms. According to a metagenomic study of the human microbiota, the majority of microbes in the human body reside in polymicrobial biofilms. According to the National Institutes of Health, biofilms are thought to be the cause of up to 80% of clinical illnesses. One of the defining traits of microbial biofilms is their resilience to antimicrobial drugs. Antimicrobial drug therapy can make microbial cells affiliated with mature biofilms up to 1000 times more tolerant/resistant than their planktonic cell counterparts. It is thought that the extracellular matrix of the biofilm, which serves as a physical barrier to the antibiotic's permeation, is largely to blame for the high levels of tolerance and resistance to antimicrobial drug treatment. Recent research has demonstrated that microbial biofilms contain similar standard drug resistance mechanisms to those found in planktonic cells. Bacteria can transmit genetic material from cell to cell through transformation, conjugation, o

r transduction, a process known as horizontal gene transfer (HGT).1 In a natural transformation, constantly growing competent cells of the same species (intraspecies transformation) or a different species (interspecies transformation) take up bacterial DNA from lysed cells and incorporate it into their genomes through genetic recombination. Natural selection will keep any genetic characteristics, such as resistance to antimicrobial drugs.2 HGT significantly eases the spread of antibiotic-resistance genes between and within species of bacteria in biofilms.3 It has been demonstrated that HGT occurs more frequently in microbial biofilms (1.9 x 10-4) than in planktonic cultures (1 x 10-9), which highlights the significance of HGT for the development of antimicrobial drug tolerance in microbial biofilms. Other well-known drug resistance mechanisms were found in biofilm-embedded cells, according to recent research on high-level tolerance/resistance to antimicrobial drugs specific to biofilms. Planktonic cells frequently possess these mechanisms, and numerous bacteria have developed cunning ways to make use of one or more of these mechanisms that are unique to biofilm cells. Specifically conferring high-level biofilm resistance/tolerance to numerous antimicrobial drugs in *Pseudomonas aeruginosa*, BrlR is a transcriptional regulator of the multidrug transporters.

A major contributor to the contamination of medical equipment and the development of microbial and chronic illnesses in the body is bacterial biofilm (Figure.1). Since they cause severe infections and have characteristics that make them resistant to antimicrobial drugs, biofilms are the cause of several human disorders. The biofilms of commensal bacteria like *Staphylococcus epidermidis*, which can inhibit the colonization of possibly pathogenic bacteria through the stimulation of host-cell immune defenses and the prevention of adhesion, are one example of a beneficial impact.

A biofilm is an organized microbial population that is adhered to a surface. In healthcare, environmental biofilms take three forms: traditional hydrated biofilms which form in wet areas such as showers, water pipes, and sinks; biofilms that form on dry surfaces such as benchtops and curtains called dry surface biofilms (DSB); and build-up biofilms (BUB) that form on surgical instruments subjected to cycles of use, decontamination (cleaning and

disinfection) and drying during storage. In addition, biofilm develops in human tissue, including persistent wounds and the lungs of cystic fibrosis patients, and biofilms on implantable medical devices result in device failure. When compared to planktonic organisms of the same species, biofilms have higher tolerances to biocides and desiccation, which is why they are important in healthcare.

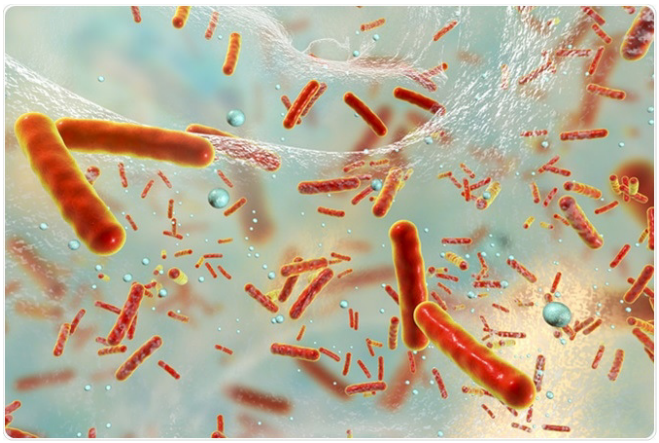

Figure.1: Biofilms in human disease: Daigrame showing the antibiotic resistance bacteria inside a biofilm (News medical).

Due to their enhanced resistance to desiccation, biofilms can endure dry conditions, which quickly kill planktonic bacteria. It has been demonstrated that DSBs are especially resistant to disinfectants and can survive for over a year on a bench without food or water. Over 90% of sterile hospital surfaces in four nations (Australia, Brazil, Saudi Arabia, and the United Kingdom) have been found to contain DSB. The effectiveness of 12 commercial disinfectants and 1000 ppm sodium hypochlorite (recommended as the disinfectant of choice by Public Health England) against DSB made up of *Candida auris* was examined by Ledwoch and Maillard in this special edition. To assess the reduction in C. auris viability, transfer of *C. auris*, and biofilm regrowth after treatment, they first created a DSB model of this emerging pathogen. They then used this model DSB in a modified ASTM 2967-15 Wiperator test. *C. auris* DSB demonstrated greater tolerance to common disinfectant agents, similar to bacterial DSB.[1] The quickly growing global market for tissue engineering-related goods and biomedical devices is already at $180 billion annually, but microbial colonization is still a problem in this sector. All medical gadgets and tissue engineering constructions are susceptible to microbial infections, regardless of their sophistication. Implantation of a biomedical device is linked to 60–70% of hospital-acquired illnesses. In the US, this results in 2 million cases yearly, adding $5 billion in extra healthcare costs to the system.

In comparison to bacteria that do not form biofilms, the degree of antibiotic resistance in biofilms can be up to 5,000 times higher. One of the main elements that can decrease the penetration of antibiotics into a biofilm structure and cause antibiotic resistance is the extracellular matrix of the biofilm. Furthermore, it has been shown that the biofilm lifestyle may have an impact on the development of antibiotic resistance. It has been demonstrated that adding a tiny electrical current to the liquid encircling a biofilm, along with small doses of antibiotics, can lower levels of antibiotic resistance in bacteria that are not part of biofilms. The bioelectric effect is the name for this. A biofilm may separate from its surface if a tiny DC current is applied on its own. A study revealed that the bioelectric effect was unaffected by the sort of current used. Many bacterial species naturally use the process of biofilm development. This is a component of their ability to adapt to their surroundings and a survival strategy. Unfortunately, the development of bacterial biofilms affects both businesses and

human health. The bacteria cell manages its survival by acquiring the resistant genes via various pathways and processes to adjust to its environment when an antimicrobial substance is used as a treatment intervention. Antibiotic use to address bacterial infections brought on by biofilms will result in increased biofilm community resistance activity as well as toxic effects on the host system. The researcher may be able to identify an effective chemical or compound that can interact with or degrade the bacterial biofilm with the aid of a thorough knowledge of the biofilm structure organization and the key chemical involved. To lessen the effect of bacterial biofilm on human health and the healthcare sector, alternative techniques or therapies must be investigated.

LITERATURE REVIEW

Microbial aggregates contained in a matrix that is attached to a biological or nonbiological surface are called biofilms. The formation of biofilms is a major issue in the food, medical, and marine industries and can have serious negative effects on both human health and the economy. The complex microbial community of a biofilm imparts persistent survival that is difficult to remove and is highly resistant to antibiotics and sanitizers. The traditional methods for battling biofilms include mechanical and/or physical removal, chemical removal, and the use of antimicrobials, sanitizers, or disinfectants to eradicate the organisms that form biofilms. Contrary to planktonic cells, biofilms are very impervious to these strategies. Therefore, new strategies that differ from traditional ones are desperately required. To address the biofilm issue for the improvement of healthcare, food safety, and industrial processes, we discuss current and new advanced antibiofilm strategies that are superior to the conventional approach in this review [2].

A population of microorganisms that are surface-attached (sessile) and that are growing inside of an extracellular polymeric matrix is known as a biofilm. These biofilm communities can be found in commercial, natural, and medical settings. They can also be cultivated in vitro for a variety of biotechnological uses. In particular, diseases linked to inert surfaces, such as medical devices for internal or external use, are diseases that biofilms play a major role in the transmission and persistence of in humans. Due to their superior defense against macrophages and antibiotics compared to free-living cells, biofilm infections on implants or in-dwelling devices are challenging to eliminate, often resulting in fatal outcomes. New methods for preventing and spreading biofilm-related infections have been made possible by recent advances in nanotechnology, and one such method may be used to treat infections that are not drug-related [3]. Biofilms are organized microbial populations that are affixed to different kinds of surfaces. Slimy extracellular polymeric substances (EPSs), which are secreted by the microbes that make biofilms, give those structures their resistance to antibiotics. Biofilms have several benefits and drawbacks. First, let's look at the drawbacks of biofilms: they increase maintenance costs and reduce total plant yields by interfering with critical processes like heat and mass transfer, fluid dynamics, and bio-corrosion. Additionally, bio-corrosion raises the potential for bacterial adhesion and contamination of dairy, brewing, and processed food items. Biofilms clog cages and obstruct nutrient inflows, which have an impact on the aquaculture and seafood sectors. Infections caused by the insertion of tubes, catheters, and valves as well as surgical procedures are just a few of the detrimental effects that biofilms have on the medical sector. Taking into account the advantages of biofilms, we observe that the careful application of biofilms can offer answers to contemporary issues. Along with treating oil spills, they are useful for the bioremediation of land and groundwater. They offer mining companies cost-effective options in the form of bioleaching and biofilm-based bioreactors for the disposal of municipal and industrial waste.

The treatment of contaminated water and the use of biofilms as biosensors for the fast and accurate detection of chemicals are both possible [4].

New difficulties have arisen as a result of the expansion of technology and the demand for different commodities on a global scale. Biofilms are collections of microbial cells that pollute and damage environmental and industrial components. These microorganisms, which have extracellular polymeric materials, colonize both living and nonliving surfaces and are a serious issue for all industries because they interfere with their processes, lower product quality, and cause financial loss. Biofilm formation can occur in sectors like the medical, food, beverage, dairy, wine, maritime, and electricity industries. Plant operation is hampered by pipe blockages, waterlogging, and reduced heat transfer effectiveness. Because they are unaware of this risk, many businesses do not implement corrective measures to control biofilm formation. Industries use a variety of conventional techniques in their everyday operations to manage these biofilms, but these are only short-term fixes. This necessitates more investigation into biofilm remediation and industrial component management. This review paper discusses biofilm issues and suggests remedies for different industrial parts. By addressing the issues with environmental biofilms, nanotechnology offers to provide several solutions and introduce a new element to the industrial economy [5].

The Journal of Industrial Microbiology's two-issue special section on microbial biofilms' success is evidence of how quickly this method of growth is becoming understood for its singularity and significance. Because of how broadly applicable the biofilm idea is, authors from almost every branch of microbiology including medical, dental, agricultural, industrial, and environmental have contributed to these two issues. Some time ago we reasoned that bacteria cannot possibly be aware (sic) of their precise location, in terms of this spectrum of anthrocentric subspecialties, and that their behavior must be dictated by a standard set of phenotypic responses to environmental conditions which must seem to them (sic) to be a continuum of very similar aquatic ecosystems. In this overview, I will, therefore, stress the common features of microbial biofilms that we should bear in mind as we use this simple universal concept to seek to understand bacterial behavior in literally hundreds of aquatic ecosystems traditionally studied by dozens of subspecies of microbiologists reared in sharply different scientific and academic conventions [6].

In synthetic biology, biological organisms are engineered using modular and generalizable designs with the final aim of creating helpful solutions to practical issues. Bacterial biofilms are one such issue; they play a significant role in the pathogenesis of many clinically significant illnesses and are challenging to eradicate due to their resilience to antimicrobial therapies and host immune system removal. We developed a bacteriophage to express a biofilm-degrading enzyme during infection to target both the bacterial cells in the biofilm and the biofilm matrix, which is made up of extracellular polymeric substances, to solve this problem. We demonstrate that compared to nonenzymatic bacteriophage treatment, this two-pronged enzymatic bacteriophage strategy considerably more effectively removes biofilm. About two orders of magnitude better than nonenzymatic phage, our engineered enzymatic phage significantly decreased bacterial biofilm cell counts by 4.5 orders of magnitude (99.997% removal). This study shows the viability and advantages of using synthetic biology to address a significant medical and industrial issue by reducing bacterial biofilms using engineered enzymatic bacteriophage [7].

The communities of microbes known as biofilms can be found in commercial, natural, and medical environments. In reality, microbes are likely to grow most frequently in biofilms in the majority of environments. A few distinctive traits are present in mature biofilms. An extracellular matrix that gives the community of biofilm microbe’s structure and protection is

usually present around them. Additionally, the design of microbes developing in a biofilm is distinctive, typically consisting of macrocolonies (hundreds of thousands of cells) encircled by fluid-filled channels. Microbes that have grown in biofilms are infamous for being resistant to a variety of antimicrobial substances, including therapeutically important antibiotics. The microtiter dish assay, which has mainly been used to study bacterial biofilms but has also been used to study the formation of fungal biofilms, is a crucial tool for the study of the early stages of biofilm formation. The mature biofilms usually connected with flow cell devices cannot be formed in this assay because static, batch-growth conditions are used. However, the assay has been successful in finding numerous elements necessary for the start of biofilm formation, including genes that are involved in the production of extracellular polysaccharides as well as flagella, pili, adhesins, and enzymes involved in the binding and metabolism of cyclic-di-GMP. Additionally, according to published research, biofilms grown in microtiter plates do acquire characteristics of mature biofilms, including an ability to withstand antibiotics and immunity-suppressing agents.

This straightforward microtiter dish test enables the development of a biofilm on the side or bottom of the dish. The assay's high throughput properties make it suitable for genetic screening as well as evaluating the formation of biofilms by different strains under various growth conditions. Numerous microorganisms, including but not limited to *pseudomonads*, *Vibrio cholerae*, *Escherichia coli*, *staphylococci*, *enterococci*, *mycobacteria*, and fungi, have been subjected to variations of this assay to evaluate early biofilm development. We will concentrate on using this assay to research biofilm formation by the model organism *Pseudomonas aeruginosa* in this procedure. The amount of biofilm development is assessed in this test using the dye crystal violet. (CV). However, several additional colorimetric and metabolic stains have been described for the microtiter plate assay to quantify the formation of biofilms. Microtiter plate assays are essential tools for the research of biofilms because of their simplicity, affordability, and adaptability [8]. Although relatively little is known about the physiology of the microorganisms involved or the mechanics of biofilm formation, the industry is well aware of the serious issues and negative economic impacts that corrosion and restricted fluid movement that biofilms can cause. Improved the way their negative effects were managed and even encouraged the development of apps that took advantage of their special qualities [9]. Microbiologists are now recognizing that bacteria frequently congregate in biofilms in nature, clusters of microbes encased in slime and adhered to a surface, after concentrating on free-floating bacteria for decades. Because these hardy microbial communities can withstand antibiotics and the immune system, biofilms can clog pipes, contaminate medical devices, and occasionally even kill people [10].

CONCLUSION

The majority of pathogenic bacterial species use biofilm formation as a protective method of growth to shield themselves from the host immune system's or antimicrobials' bactericidal effects. The ability of the bacteria cells to survive by forming biofilms poses problems for the medical industry in terms of devices and diseases that are linked to biofilms. The effect of the bacterial biofilm problem is getting worse over time, and the high antimicrobial drug tolerance contributes to increased morbidity and mortality globally. This review will highlight the main characteristics of the biofilm, the issue of biofilm in clinical practice, which also covered the pertinence of the biofilm in clinical practice, device-related biofilm disease, oral disease, and the significant bacterial species involved in biofilm-related infections. Understanding the crucial function of bacterial biofilm in associated disorders will provide a fresh perspective on the most effective methods and complementary therapies for the biofilm-related disease.

REFERENCES:

[1] K. Vickery, "Special Issue: Microbial biofilms in healthcare: Formation, prevention and treatment," *Materials*. 2019. doi: 10.3390/ma12122001.

[2] M. Sadekuzzaman, S. Yang, M. F. R. Mizan, and S. D. Ha, "Current and Recent Advanced Strategies for Combating Biofilms," *Compr. Rev. Food Sci. Food Saf.*, 2015, doi: 10.1111/1541-4337.12144.

[3] S. Srivastava and A. Bhargava, "Biofilms and human health," *Biotechnology Letters*. 2016. doi: 10.1007/s10529-015-1960-8.

[4] S. K. Yadav and S. Sanyal, "Biofilms: The good and the bad," in *Biofilms in Human Diseases: Treatment and Control*, 2019. doi: 10.1007/978-3-030-30757-8_2.

[5] V. Vishwakarma, "Impact of environmental biofilms: Industrial components and its remediation," *Journal of Basic Microbiology*. 2020. doi: 10.1002/jobm.201900569.

[6] J. W. Costerton, "Overview of microbial biofilms," *J. Ind. Microbiol.*, 1995, doi: 10.1007/BF01569816.

[7] T. K. Lu and J. J. Collins, "Dispersing biofilms with engineered enzymatic bacteriophage," *Proc. Natl. Acad. Sci. U. S. A.*, 2007, doi: 10.1073/pnas.0704624104.

[8] G. A. O'Toole, "Microtiter dish Biofilm formation assay," *J. Vis. Exp.*, 2010, doi: 10.3791/2437.

[9] S. A. Blenkinsopp and J. W. Costerton, "Understanding bacterial biofilms," *Trends Biotechnol.*, 1991, doi: 10.1016/0167-7799(91)90045-J.

[10] C. Potera, "Biofilms invade microbiology," *Science*. 1996. doi: 10.1126/science.273.5283.1795.

CHAPTER 5

FUNCTIONALROLE OF BIOFILMS IN THE FOOD INDUSTRY

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ABSTRACT:

The capacity of microorganisms to attach and grow on food and food-contact surfaces under favorable conditions is particularly significant. Different processes play a role in the attachment and growth of biofilms, which is a dynamic process. Microorganisms attach to and colonize surfaces in touch with food in part due to extracellular polymeric substances. Different methods have been used to properly research and comprehend biofilm attachment and control. If the microorganisms from surfaces in contact with food are not fully eliminated, they may cause the formation of biofilm and also increase the potential for biotransfer. To avoid biofilm formation on surfaces that come into contact with food, a variety of preventive and control strategies, including hygienic plant layout and design of equipment, choice of materials, correct use, and selection of detergents and disinfectants, can be effectively implemented. Bacteriocins and enzymes are also becoming more significant and have a special potential in the food business for the removal of biofilms and effective biocontrol. These more recent biocontrol techniques are thought to be crucial for maintaining biofilm-free systems, as well as for food purity and safety. This chapter on biofilms has garnered a lot of attention in the context of the role of biofilms in the food industry.

KEYWORDS:

Biofilms formation, Biofilms food, Fungal Spore,Food processing, Microorganism food.

INTRODUCTION

Wet food preparation environments and food matrixes with water activities above 0.9 are havens for the growth of microorganisms and biofilms. Biofilms are considered of great concern regarding the functioning of mechanical parts that may be blocked, energy consumption, which becomes higher when heat transfer decreases, and corrosion as the corrosion rate of surfaces increases underneath biofilms (corrosion grows 10–1000 times faster causing loss of material and increasing porosity) but their presence in food and food processing environments is also a serious public health risk due to problems associated with foodborne illnesses and food spoilage. Some pathogenic bacteria, including *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, and *Pseudomonas aeruginosa*, as well as toxic bacteria, including *Staphylococcus aureus* and *Bacillus cereus*, create the biofilms that pose a danger to the safety of food products. Such bacteria persist in food preparation environments and recontaminate processed foods as a result of biofilms. Recalls are required when food items become contaminated. These actions impose a heavy financial burden on the business and also harm brands [1].

Bacillus Cereus A spore-forming Gram-positive anaerobic or facultative anaerobic bacterium called Bacillus cereus can grow in a variety of environments at a broad range of temperatures (4 C to 50 C) (Figure.1A-1F). It is immune to radiation, thermal treatment, and chemicals. B. cereus is a soil-dwelling organism that is frequently kept away from food and food-related products like rice, dairy products, veggies, and meat. It releases toxins that can make people ill and have diarrhea. On surfaces that come into touch with food, like storage tanks, conveyor belts, and stainless steel pipes, B. cereus is in charge of forming biofilms. It can

also create floating or submerged biofilms that produce a wide range of bacteriocins, metabolites, surfactants, and enzymes like proteases and lipases that can alter the sensory qualities of food. Bacterial flagella's mobility provides access to surfaces that are good for biofilm formation, and biofilms must proliferate on uncolonized surfaces. *B. cereus* flagella, however, have not been discovered to be directly associated with adhesion to glass surfaces, but their motility can play a significant part in biofilm formation.

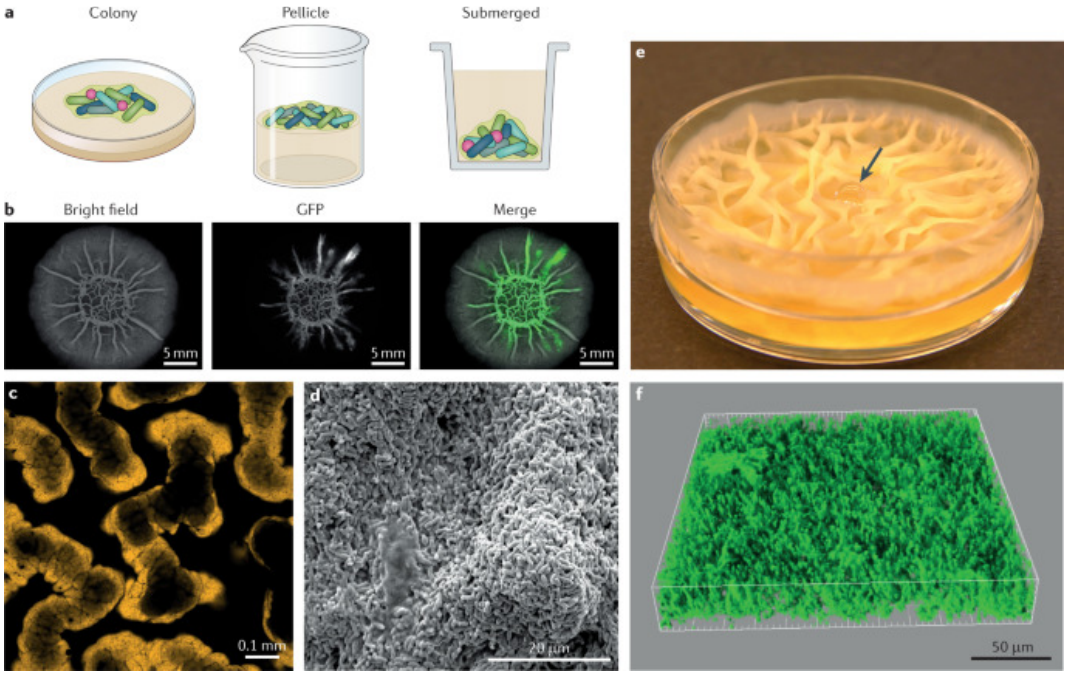

Figure 1: Bacillus spp: Diagramed showing the bacillus species biofilms formation (Nature).

Campylobacteria are very contagious. The number of cells that *C. jejuni* can infect varies from 500 to 10,000, depending on the strain, the degree to which environmental stresses cause cell damage and the host's susceptibility. In most cases, only the mesophilic C. fetus is intrusive. Occasionally invading thermophilic species, like *C. jejuni*, have an optimal temperature of 42°C. Meningitis, pneumonia, miscarriage, and a severe type of Guillain-Barré syndrome are some of the infections' symptoms. Patients have provided C. fetus strains that are thermotolerant and thrive at 42°C. Campylobacters are corkscrew-moving, microaerophilic, very tiny, curved, thin, Gram-negative rods (1.5–5 m). They frequently combine to create zigzag patterns. Many different types of wild and household animals, particularly birds, carry campylobacters in their gastrointestinal tracts. They can cause illness in people as well as a transient asymptomatic carrier state. This is particularly common in underdeveloped nations. *C. jejuni* can live for 2-4 weeks in moist, low-oxygen environments at 4°C, frequently outlasting the product's shelf life.

In addition, they can endure -20°C for 2 to 5 months, but only a short time at ambient temperature. Environmental stresses that most bacteria don't experience, like air exposure, drying, low pH, heating, freezing, and extended storage, cause more cell damage and make recovery more difficult. Stressed-out and older organisms progressively develop coccidia and become more challenging to culture. Recovery can be greatly aided by oxygen-reducing media components like hemin and charcoal, a microaerobic environment, and pre-enrichment. 70% of Campylobacter-related diseases each year are caused by consuming food and water contaminated with untreated animal or human waste. Unpasteurized milk, meats, poultry, shellfish, fruits, and veggies are on the list of items.

Biofilms are firmly attached to numerous surfaces used in the food business and contain microbial cells that are shielded by a self-produced matrix. This defense makes microorganisms in biofilms much more difficult to get rid of and thus regulate than cells suspended in suspension. *Listeria monocytogenes* is a bacterium that frequently creates these structures and survives in food preparation facilities. Since there doesn't seem to be clear guidance on how to manage the risk that the bacteria presents, numerous efforts have been made to develop control strategies that can be used in the food industry (Figure.2).

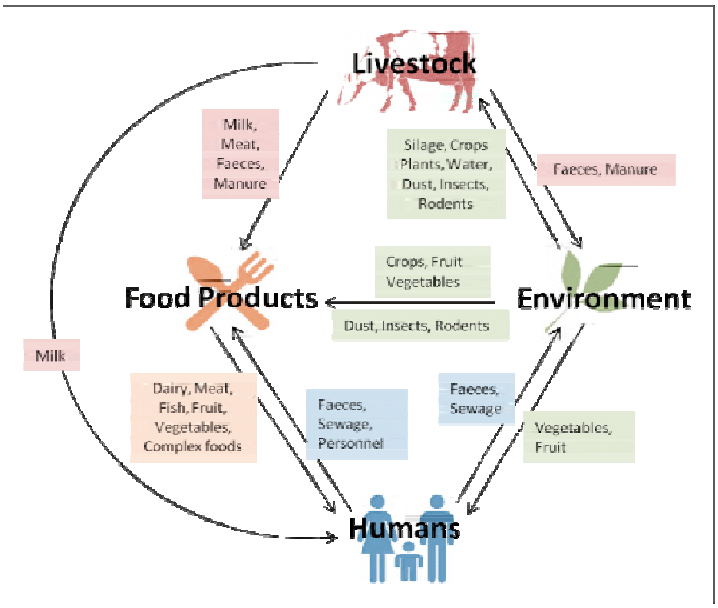

Figure 2: *Listeria monocytogenes*: Diagramed showing the presence of the *Listeria monocytogenes in* different places (Research gate).

The strategies for the control of this pathogen rely on the type of surface, the nature of the product, the circumstances of the food industry environment, and even the budget, as there is no standardized procedure that is applied uniformly to all food sectors. Different preventive and corrective actions are taken by the food business on potential *L. monocytogenes*-contaminated surfaces. To determine whether the therapy can be sustained over time, a critical assessment of the sanitization techniques used must be made. The strategies presently in use to get rid of biofilms and prevent their growth in processing facilities for various food types (such as dairy, meat, seafood, chilled vegetables, and ready-to-eat products) will be the main focus of this review [2]. A species of rod-shaped (bacillus) Gram-negative bacteria belonging to the Enterobacteriaceae family is called Salmonella. *Salmonella enterica* and *Salmonella bongori* are the two types of Salmonella. The type species is *S. enterica*, which is further split into six subspecies with a total of more than 2,600 serotypes. Daniel Elmer Salmon (1850–1914), an American veterinarian, received the moniker Salmonella.

Salmonella species are non-spore-forming, primarily motile enterobacteria with peritrichous flagella and cell sizes between 0.7 and 1.5 m. (all around the cell body, allowing them to move)[5]. They are chemotrophs, drawing energy from organic substances through oxidation and reduction processes. Additionally, they are facultative anaerobes, able to produce ATP either anaerobically (without oxygen) or aerobically (with oxygen) depending on the available electron acceptors. Foods like poultry, beef, pork, eggs, fruits, veggies, and even processed foods can contain salmonella. Some individuals are more susceptible to infections and serious illnesses.

A *Staphylococcus aureus* is a Gram-positive, facultatively anaerobic, non-spore-forming, non-motile bacteria that can produce enterotoxins between 10-46 °C. S. aureus is a serious problem in food factories because it can grow on the skin and mucous membranes of people who touch food. These heat-stable enterotoxins can be released during the development of S. aureus in foods tainted by food handlers. The bacterium thrives in foods with high salt or sugar concentrations but low water activity. Meat and meat products, chicken and egg products, milk and dairy products, bakery products, salads, and especially cream-filled cakes and pastries and sandwich fillings are among the foods commonly linked to Staphylococcal food-borne illness. Numerous enteric toxins produced by S. aureus are well recognized. These enterotoxins cause the T-cells' class II MHC (major histocompatibility complex) to attach to them, activating them and potentially causing acute toxic shock syndrome with sickness and diarrhea.

Pseudomonas is a rod-shaped, motile, heterotrophic, Gram-negative bacteria. *Pseudomonads* are typically ubiquitous psychrotrophic spoilage organisms that can be found in low-acid dairy products, on the surfaces of fruits, veggies, and meat, as well as in the floors and drains of food processing facilities. Motile microorganisms generate extracellular filamentous appendages, which have distinct effects on the attachment process and surface interaction. Flagella and pili have undergone extensive study. *Pseudomonas aeruginosa,* which is 1–5 m long and 0.5–1.0 m broad, can be used as a model organism when discussing how biofilms form and are controlled by quorum sensing. With nitrate serving as the final electron acceptor, a facultative aerobe develops through both aerobic and anaerobic respiration. Massive quantities of EPS are produced by *Pseudomonas* species, which are also known to adhere to and create biofilms on stainless steel surfaces. They can co-exist in biofilms with other bacteria to create multispecies biofilms, which increase their stability and resistance. A distinct blue discoloration (pyocyanin) on fresh cheese generated by *P. fluorescens* can accompany these biofilms.

A thermophilic, aerobic bacteria called *Geobacillus stearothermophilus* (previously known as *Bacillus stearothermophilus*) creates heat-resistant spores. In dairy facilities, it creates biofilms that adhere to stainless steel surfaces. Although the bacterium has no impact on public health, its prevalence in ingredients, canned foods, and milk powders is noteworthy. It is the typical species that causes low-acid canned goods to spoil in a thermophilic flat sour manner. Food spoilage can be prevented by properly handling canned foods at temperatures below the bare minimum needed for development. The thermostable enzymes produced by *Geobacillus stearothermophilus* have a broad range of industrial uses.

Anoxybacillus flavithermus, number another gram-positive, thermophilic, spore-forming, facultatively anaerobic, and non-pathogenic bacterium is *Anoxybacillus flavithermus*. Low concentrations of the thermophilic spore-forming bacteria that exist in farm environments can be detected in raw milk. They can form during the production of dairy powders and can be found in large concentrations. They are resistant to pasteurization treatments used in the industry. An evaluation of the species diversity of thermophilic spores was conducted on 61 samples obtained from industrial sites to assess the contamination of dairy powders made in France. This enabled the identification of a wide variety of spore contaminants. It appears that the three thermophilic spore-forming bacterium species most frequently found in dairy powders are *A. flavithermus*, *G. stearothermophilus*, and *B. licheniformis*. The variety of dairy powders investigated affects these prevalence rates. Concerning the enzymatic activity, A. flavithermus, *G. stearothermophilus*, and *B. licheniformis* in particular, have been shown to have a propensity for spoilage due to phenotypic diversity. As a consequence, tainted powders that are used in food formulations as ingredients could spoil food. A detailed

analysis of the manufacturing process of dairy powders, associated with the growth capacities of thermophilic spore-forming bacteria, allowed us to evaluate the steps of the manufacturing process of the powders favoring the development, the formation of biofilms and spores of the studied species. Finally, the estimations of the capacity of biofilm formation by *A. flavithermus* and *G. stearothermophilus* on the one hand, and of their resistance to the cleaning treatments, on the other hand, revealed that the *A. flavithermus* species is the most resistant to the alkaline treatments whereas the resistances to the acid treatments seem to be similar for both species. The findings of this thesis have made it possible to assess the potential for thermophilic spore-forming bacteria to grow in the dairy powder industry and to better understand the controls over both their growth and eradication.

Additionally, numerous brewers in Germany, Spain, Norway, Japan, the Netherlands, Sweden, and France have isolated *P. cerevisiiphilus*. For instance, in the fishing industry, mixed pathogenic species like *Aeromonas hydrophila*, *L. monocytogenes*, *S. enterica*, or Vibrio spp. can create biofilms on fresh fish products, posing serious health and financial risks. In order to create mixed biofilms, E. coli interacted with *Burkholderia caryophylli* and *Ralstonia insidiosa* in a fresh-cut produce preparation facility.

Investigating mixed species of biofilms, such as Candida albicans, has led several authors to discover beneficial synergies in other research. Biofilm-related impacts (pathogenicity, corrosion of metal surfaces, and modification of organoleptic properties as a result of protease or lipase secretion) are extremely significant in the food industry. Pipelines, raw milk tanks, butter centrifuges, pasteurizers, cheese tanks, and packing tools, for instance, can serve as surface substrates for biofilm formation at various temperatures and contain a variety of mixed colonizing species. To prevent contamination and guarantee food safety in the food business, it is crucial that precise methods to visualize biofilms in situ be established [1].

The creation of online tracking techniques to track the adhesion, development, and/or removal of deposits and biofilms from surfaces in an industrial setting lowers the cost of cleaning operations and minimizes production pauses for upkeep. Traditional techniques for biofilm detection, like agar plating, are ineffective because it is challenging to cultivate many biofilm microorganisms. This is because some foodborne pathogens, like L. monocytogenes, have the ability to penetrate the body in a form known as "viable but nonculturable" (VBNC), which has a low metabolic activity. By using cultivation techniques, it is impossible to identify these VBNC cells, which may even help cells survive in stressful situations like low temperatures. For instance, PCR amplification can be used to identify VBNC cells. As a result, the creation of novel methods for identifying the production of biofilms is given significant weight. Metagenomics and metatranscriptomics are two additional cutting-edge techniques for biofilm identification studies that can illuminate the intricate relationships within a biofilm community. The creation of novel methods to identify the formation of biofilm in industrial settings has thus received significant attention, as the majority of biofilm online monitoring techniques are currently dependent on the introduction of an external perturbation to the system [3].

LITERATURE REVIEW

In numerous environments, microbial surface colonization (biofilms) has been recorded. Biofilms may be a source of contamination in settings used for food processing, according to a recent study. This overview will go over some historical aspects of biofilms, potential mechanisms for bacterial adhesion to surfaces, biofilm research techniques, and potential issues adherent microorganisms could have with food processing [4].

This review investigates how biofilm-forming microbial communities affect food quality and safety in settings where food is processed. Both pathogenic and spoilage microorganism- and microbe-produced biofilms, which are particularly important in the processing of fermented foods, are the subject of this paper. The variability of biofilm formation within a species, relationships between species within a biofilm that is cooperative or competitive, variables affecting the ecology and architecture of biofilms, and potential effects on removal are all covered in this paper. It is described how certain food components and various environmental factors that frequently exist during food processing affect the ability of biofilm to form. We investigate the tools that are available for observing and characterizing wild microbial biofilms in situ in food processing plants. Finally, a summary of a study on novel agents or techniques for the prevention or removal of biofilms is provided [5].

Research on wastewater purification, dental plaques, and water distribution networks were the only areas of biofilms that attracted attention in the past. In recent years, biofilm has gained popularity as a research subject in a variety of other fields, including food safety. By transferring detached organisms to different parts of processing facilities, biofilm formation can jeopardize the cleanliness of food surfaces and environmental surfaces. These detached organisms, which are more resilient to various stressors and microbial inactivation, including some food preservation techniques, are unfortunately not comparable to typical microorganisms suspended in an aquatic habitat. Different types of microscopic techniques unveiled the intricate microstructures of biofilms, which are made up of numerous symbiotic organisms, some of which are human pathogens. The creation of biofilms, their importance on food or food contact surfaces, their capacity to shield foodborne pathogens from environmental stresses, and current approaches for studying biofilms on food contact surfaces were all covered in this paper [6].

Microorganisms can withstand extreme changes in their surroundings even though they are constantly at war with one another. The cells in this setting must adjust to the circumstances or perish. The majority of cells hide under a layer of polysaccharides after adhering to a surface to live. This eventually develops into a biofilm where various bacteria can coexist. Even though the environment is not optimal, the microorganisms in the biofilm create their own microenvironment in which their species can survive. The biofilm serves as a trap for obtaining nutrients in addition to shielding from hostile surroundings. In many areas of the food business, biofilm formation creates issues because it can waste energy, reduce flow and heat transmission, or obstruct membrane pore openings. Biofilms can be created by both virulent pathogenic bacteria and benign microbes. Pieces of this biofilm may separate and disperse in the process flow as a result of flow fluctuations or cleaning [7].

Almost any spot in the food chain can allow Listeria monocytogenes to enter. Environments used in food preparation, however, appear to be particularly significant. Food processing facilities are microbial habitats from an ecological perspective, and cleaning and sanitizing processes continually disturb these habitats. Even though *L. monocytogenes* are thought to be present everywhere in nature, it is significant to note that not all strains of the organism seem to be dispersed equally; rather, the distribution of the organism appears to be linked to particular habitats. There is currently no proof that L. monocytogenes-associated biofilms have contributed to food contamination or foodborne outbreaks, possibly as a result of the lack of biofilm isolation and identification during outbreak investigations or the ambiguity surrounding the meaning of biofilm. We propose that contamination patterns may be investigated in the context of how the environment within food processing plants affects biofilm formation because L. monocytogenes is known to colonize surfaces.

The ability of a lineage to form biofilms in particular ecological niches will be addressed in this review along with direct and indirect epidemiological and phenotypic evidence. A critical viewpoint on the evolution of the biofilm idea is presented, concentrating on the applications, benefits, and drawbacks of the existing definitions. It has been suggested that biofilm development might serve as a different proxy for microbial fitness [8].

Listeria monocytogenes has long been regarded as a foodborne pathogen of significance to public health and of special concern for high-risk population groups due to high mortality and hospitalization rates. Because *L. monocytogenes* is so common, it is difficult for food manufacturers to keep it out of places where food is produced (FPEs). Additionally, the ability of L. monocytogenes strains to colonize FPEs may cause L. monocytogenes to be repeatedly found during FPE monitoring. Food product contamination that necessitates a recall places a significant financial strain on the business, which is made worse by harm to the brand. Listeria hotspots and biofilms may form as a result of poor equipment design, building architecture, worn or damaged equipment, and situations where conventional cleaning and disinfecting techniques may not be effective. Innovative biocontrol techniques may provide FPEs with efficient ways to enhance L. monocytogenes control and reduce cross-contamination of food.

Since they have the capacity to infect and kill particular bacteria, bacteriophages have long been used in medicine. Endolysins, bacteriophage hydrolytic enzymes that break down Gram-positive bacteria's cell walls, are being studied as a biocontrol strategy for food preservation as well as for use in nanotechnology and medical uses. Bacteriocins, which are antibacterial proteins, have been used as an option to antibiotics for biopreservation and extending the shelf life of food products. Essential oils are naturally occurring antimicrobials produced by plants. They have long been used as food additives and preservatives, and more recently as a way to stop microbes from causing food to spoil. Bacteria in the environment typically exclude one another through competition. A possible biocontrol application, however, is the deliberate selection and application of bacteria to cause the competitive exclusion of foodborne pathogens. This review discusses these cutting-edge biocontrol techniques and how they can be used to keep food safe and avoid spoilage. It also looks at how well they might be able to control L. monocytogenes in biofilms that form in food production facilities [9].

The topic of biofilms has garnered a lot of attention in the context of food safety. The capacity of microorganisms to attach and grow on food and food-contact surfaces under favorable conditions is particularly significant.

Different processes play a role in the attachment and growth of biofilms, which is a dynamic process. Microorganisms attach to and colonize surfaces in touch with food in part due to extracellular polymeric substances. Different methods have been used to properly research and comprehend biofilm attachment and control. If the microorganisms from surfaces in contact with food are not fully eliminated, they may cause the formation of biofilm and also increase the potential for biotransfer.

To avoid biofilm formation on surfaces that come into contact with food, a variety of preventive and control strategies, including hygienic plant layout and design of equipment, choice of materials, correct use, and selection of detergents and disinfectants, can be effectively implemented. Bacteriocins and enzymes are also becoming more significant and have a special potential in the food business for the removal of biofilms and effective biocontrol. These more recent biocontrol techniques are thought to be crucial for maintaining biofilm-free systems, as well as for product quality and safety [10].

CONCLUSION

This chapter presents the ability of some microorganisms (*Listeria monocytogenes*, *Escherichia coli, Salmonella enterica, Campylobacter jejuni, Pseudomonas aeruginosa*) and toxigenic bacteria (Bacillus cereus, Staphylococcus aureus) to form biofilms and contribute to the persistence of these microorganisms in the food industry.

Genes implicated in biofilm production are along with particularities regarding attachment and composition of biofilms formed in food and food processing environments. Because they have the ability to serve as a persistent source of microbial contamination that may cause food spoilage or disease transmission, biofilms produced in food-processing environments are particularly significant. Why is it crucial to stop bacteria from forming during food processing.

For instance, biofilm found in food processing facilities can secrete toxins. From there, they can contaminate a food matrix, resulting in intoxications for one person or many people (in the event of an epidemic).

In either scenario, the existence of biofilms in a food manufacturing facility endangers people's health. The intercellular signaling system, the cyclic nucleotide second messenger, and biofilm-associated proteins are just a few of the survival mechanisms developed by biofilms to avoid being destroyed by disinfectants and the real danger they pose to the food industry.

REFERENCES:

[1] L. Grigore-Gurgu, F. Ionela Bucur, D. Borda, E.-A. Alexa, C. Neagu, and A. Ioana Nicolau, “Biofilms Formed by Pathogens in Food and Food Processing Environments,” in *Bacterial Biofilms*, 2020. doi: 10.5772/intechopen.90176.

[2] T. Mazaheri, B. R. H. Cervantes-Huamán, M. Bermúdez-Capdevila, C. Ripolles-Avila, and J. J. Rodríguez-Jerez, “Listeria monocytogenes biofilms in the food industry: Is the current hygiene program sufficient to combat the persistence of the pathogen?,” *Microorganisms*, 2021, doi: 10.3390/microorganisms9010181.

[3] S. Galié, C. García-Gutiérrez, E. M. Miguélez, C. J. Villar, and F. Lombó, “Biofilms in the food industry: Health aspects and control methods,” *Frontiers in Microbiology*. 2018. doi: 10.3389/fmicb.2018.00898.

[4] S. K. Hood and E. A. Zottola, “Biofilms in food processing,” *Food Control*. 1995. doi: 10.1016/0956-7135(95)91449-U.

[5] A. Alvarez-Ordóñez, L. M. Coughlan, R. Briandet, and P. D. Cotter, “Biofilms in Food Processing Environments: Challenges and Opportunities,” *Annual Review of Food Science and Technology*. 2019. doi: 10.1146/annurev-food-032818-121805.

[6] F. j. P. Elortondo, J. Salmerón, M. Albisu, and C. Casas, “Biofilms in the food industry,” *Food Sci. Technol. Int.*, 1999, doi: 10.1177/108201329900500102.

[7] L. V. Poulsen, “Microbial Biofilm in Food Processing,” *LWT*. 1999. doi: 10.1006/fstl.1999.0561.

[8] W. B. Valderrama and C. N. Cutter, “An Ecological Perspective of Listeria monocytogenes Biofilms in Food Processing Facilities,” *Crit. Rev. Food Sci. Nutr.*, 2013, doi: 10.1080/10408398.2011.561378.

[9] J. A. Gray, P. S. Chandry, M. Kaur, C. Kocharunchitt, J. P. Bowman, and E. M. Fox, "Novel biocontrol methods for Listeria monocytogenes biofilms in food production facilities," *Frontiers in Microbiology*. 2018. doi: 10.3389/fmicb.2018.00605.

[10] C. G. Kumar and S. K. Anand, "Significance of microbial biofilms in food industry: A review," *International Journal of Food Microbiology*. 1998. doi: 10.1016/S0168-1605(98)00060-9.

CHAPTER 6

ROLE OF BIOFILMS IN THE WASTEWATER TREATMENT

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ABSTRACT:

Wastewater is treated using biofilm, which interacts with liquid oxygen, ammonia, nitrogen, and biological oxygen demand (BOD). Wastewater nutrients encourage the development of microbes and the metabolites produced by these organisms, which are then used to remove contaminants from the wastewater. Systems for biologically treating wastewater are crucial for enhancing both human health and water purity. Thus, this chapter quickly discusses various particularly biofilm technologies, the formation of biofilms the factors influencing their formation, as well their structure and function. To conduct a thorough investigation of the composition, diversity, and dynamics of biofilms, it also takes on a variety of traditional and contemporary molecular methods. The performance, reliability, and stability of biofilm-based wastewater treatment technologies must be improved with the help of these statistics.

KEYWORDS:

Biofilms Formation, Biofilm Development, Biofilms Growth, Treatment System, Water Waste Treatment

INTRODUCTION

Water is a fundamental necessity, but only about 1% of it is available for human use. Rapid population growth, climatic change, environmental pollution, urbanization, industrialization, and contamination of existing water sources are all contributing factors to the current worldwide water crisis. Because a large portion of the waste is discharged from industries, municipal sewers, and agricultural regions without previous treatment, the quality of freshwater in rivers and streams is impacted. The quality of groundwater is declining due to unprocessed sewage containing domestic waste along with human and animal excretion products, leading to worldwide deaths and other environmental factors, including biodiversity reduction and an increasing number of water-related infections, among others. WHO estimates that water pollution causes about 30% of all illnesses and 40% of deaths worldwide [1].

Microorganisms that adhere to surfaces and proliferate there form biofilms. They are held together by highly hydrated extracellular polymers (EPS, 70–95% dry weight), in which the microorganisms are embedded, and are primarily composed of water (70–95% moist weight). Adhering cells are more active and resistant to toxins than their peers in suspension. The cells create well-organized consortia while immobilized next to one another and are able to carry out sequential degradation processes. The majority of microbes on earth live in biofilms, which are pervasive. The unique characteristics of biofilms are used in bioreactor technology for environmental protection, applied to soil remediation, refuse air and water purification, and solid waste decomposition. In addition to causing metal corrosion and microbially-induced weathering of mineral materials like stone or cement, biofilms can have negative impacts. Damaged oil tanks, pipes, and concrete sewers, for example, have resulted in significant soil, groundwater, and surface water pollution.

Large amounts of biocides are used when biofilms form on heat exchangers, filter materials, and separation membranes. After use, these create issues with wastewater. In order to maximize the application of desired biofilms and reduce the negative effects of unwanted biofilms and countermeasures, it may be helpful to have a better knowledge of the specific characteristics and dynamics of biofilm development and processes [2].

The environment's circumstances, such as the surface's characteristics and the deposition of organic materials in the surface carrier, have an impact on the biofilm's complex formation process. The surface will initially be acclimated to adsorb organic molecules prevalent in the nearby environment before the microorganism attaches to it for biofilm formation. The adhesion, retention, attachment, and growth of the microorganisms to the surface come next (Figure 1). The flagella and pili-equipped motile bacteria are in charge of starting the biofilm adhesion process to the carrier substance. The movement of bacteria from their planktonic condition to the surface depends on their flagella. The early adhesion of the cells to the surface has been linked to filamentous protein complexes called type IV pili. They are reportedly essential for the development of biofilms. The absorption and distribution of the surrounding organic substance into the carrier surface are influenced by the chemical characteristics of the surface.

Additionally, the sort of organic substances that are absorbed by the surface may alter the characteristics of the surface carrier. Instead of developing as a monoculture, biofilms typically form a partnership. When a biofilm is forming, members of the same family of microorganisms communicate by exchanging chemical compounds that instruct planktonic species to move into the biofilm stage. Quorum sensing is the term for this type of bacteria communication. In the process of creating biofilms, microbes create extracellular polysaccharides (EPSs), which serve as a cover for the biomass and facilitate adhesion to surfaces. Compared to planktonic bacterial cells, biofilms are typically more immune to being killed by external contaminants.

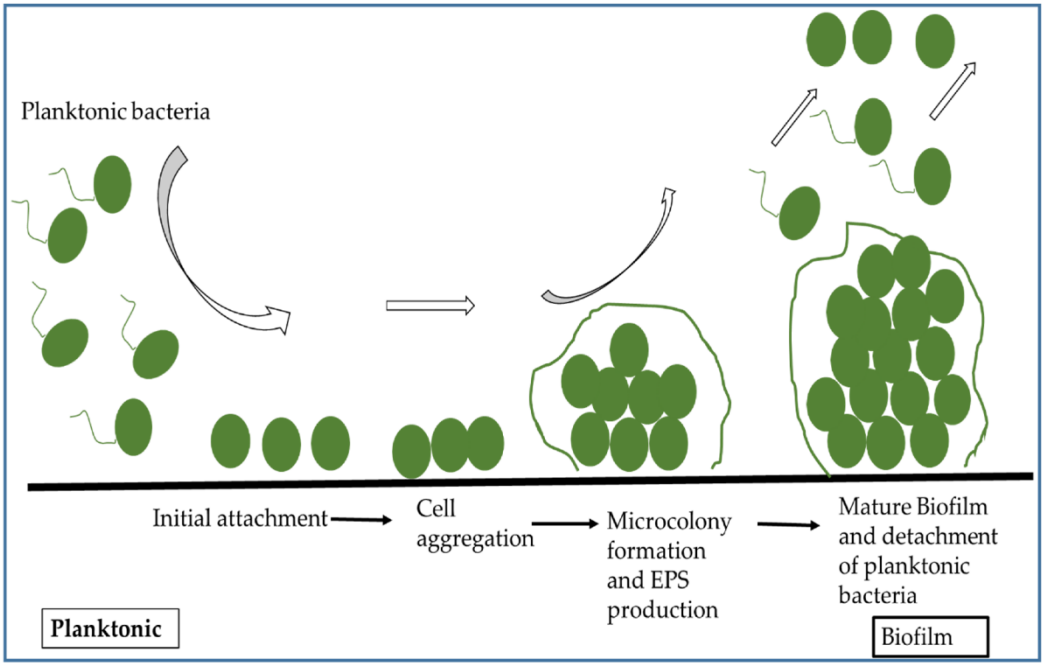

Figure 1: Biofilms growth: Diagrammed showing the step-wise growth of the biofilm for wastewater treatment (MDPI).

Biofilm system is a well-developed technology in which solid media are added to suspended growth reactors to provide attachment surfaces for biofilms, to increase the microbial concentration as well as rates of contaminant degradation biofilms to take advantage of several removal mechanisms, including biodegradation, bioaccumulation, biosorption, and biomineralization. The microbial communities in the biofilm break down various nutrients, including carbonaceous materials, substances containing phosphorus and nitrogen, and trapped pathogens from the effluent. Following the removal of pollutants, biofilter-treated water is either released into the ecosystem or used for farming and other recreational activities. (Figure .2) illustrates graphically how biofilm on the filter media removes pollutants from wastewater. Wastewater treatment with biofilm systems has several advantages, including operational flexibility, low space requirements, reduced hydraulic retention time, resilience to changes in the environment, increased biomass residence time, high active biomass concentration, enhanced ability to degrade recalcitrant compounds as well as a slower microbial growth rate, resulting in lower sludge production.

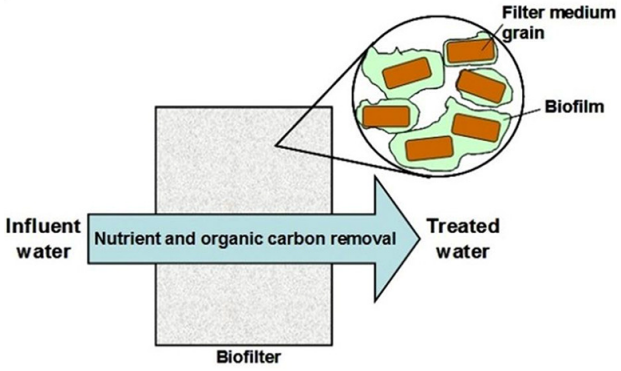

Figure 2: Utilizing biofilms: Schematic representation of biofilm on the filtration material removing pollutants from wastewater (intechopen).

Although biofilms can occasionally have negative effects, they also have a lot of potential for specific uses, such as bioremediating hazardous waste sites, biofiltering municipal and industrial water and wastewater, and creating biobarriers to prevent contamination of soil and groundwater. Biofilms are frequently advantageous when used in designed systems for the treatment of wastewater. These systems include modified lagoons, trickling filtration systems, and specialty nutrient or waste removal systems, among others. Because the microbial communities in biofilm-based treatment systems are resilient to variations in toxicity concentrations and impervious to shifting environmental circumstances, they are advantageous. Pollutants in freshwater and effluent are typically heavy metals like copper, lead, and zinc. Teitzel et al. conducted research to look at how these heavy metals affected biofilm and planktonic *P. aeruginosa*. This study showed that biofilms can be up to 600 times more robust to heavy metal stress than free-swimming cells in a rotating-disk biofilm reactor.

Tricking filters, rotating biological contactors, and various reactor types with stationary and moving beds are examples of fixed-film processes. They all rely on microbial cells adhering to an inert support medium to create a biofilm, which typically has a high specific surface area for maximum biofilm development. Though oxygen can only diffuse through biofilms a short distance before being used, leaving the deepest layers of the biofilms anoxic or anaerobic, the thickness of the biofilms is crucial for wastewater purification. Biofilms are extraordinarily complex communities that are found in the outer aerobic layers and are controlled by filamentous bacteria as well as protozoa, small metazoans, and occasionally

some vertebrates. These heterotrophic bacteria are crucial for the breakdown of wastewater's organic material. As only the biofilm's surface layer is thought to be effective in terms of oxidation, only a thin coating of film on the reactors is necessary for effective purification. Due to a lack of air, anaerobic bacteria are the most prevalent microbes in the inner layer of biofilms. According to reports, interior anaerobic biofilms contain a higher proportion of non-viable bacteria and a significantly lower density of microorganisms. Excessive biofilms on the substrate are not advised during filtration operation. This is because dense biofilm development does not result in its instability and detachment from the supports, blocking the void spaces between the medium that would otherwise permit oxygen transfer and wastewater movement without reducing treatment efficiency [3]. Several factors affect the growth of the microorganism Growth. The morphological and behavioral influences on biofilm. The development of biofilm is influenced by environmental variables such as bacterial metabolites, oxygen concentration, pH, and nutrients. The development of biofilms is favored by hydrophobic surfaces, low salinity, low temperatures, and pH values of 7-8. This chapter explains the various techniques biofilms use to treat water waste. Environments that are rich in microorganisms are a good source for treating contaminated water.

LITERATURE REVIEW

A focused study has led to the development of fundamental concepts describing biofilms. One typical application of biofilms is the treatment of urban wastewater in reactors. The mechanistic knowledge of biofilm reactors is supported by applied research. Despite the advent of mathematical models as trustworthy tools for research and practice, the empirical data derived from such applied research has been used to create design criteria for biofilm reactors and continues to serve as the foundation for biofilm reactor design. Unfortunately, there isn't much material available to fill the knowledge gap between our present knowledge of the fundamentals of biofilm and reactor-scale empirical data. As a result, the literature clearly distinguishes between micro- (biofilm) and macro- (reactor) dimensions. The divide is highlighted in this chapter. The first part of the paper discusses fundamental research and the current knowledge of biofilms. In the application-focused second section, biofilms are discussed as a method for treating urban wastewater. A basis for addressing this disconnection is presented by (1) describing the fundamental biofilm principles that can be uniformly applied to biofilms in several disciplines extending from medicine to environmental biotechnology and (2) describing a fundamentals-based approach to understanding and applying biofilms in reactors. Although mathematical biofilm models are frequently used in both study and practice, only a brief mathematical description of them is given here. Part III concludes by citing instances of undesirable biofilms in the water and wastewater sectors and discussing efforts to lessen their effects. Metals, concrete, and plastics are all susceptible to biodeterioration due to metabolic processes controlled by microorganisms found in biofilms. According to estimates, the US economy loses billions of dollars each year due to microbially influenced rusting (MIC) alone [4].

One of the major sources of pharmaceutical residue in the surface water is wastewater treatment plants (WWTPs). To investigate the effects of their discharge through the changes in biofilm composition (compared to a corresponding upstream biofilm) in terms of pharmaceutical concentrations and bacterial community modifications, epilithic biofilms were collected downstream from 12 WWTPs of different types and capacities. (Microbial diversity and resistance integrons). A potential indicator for assessing the effects of WWTPs on the aquatic environment nearby is the biofilm. The use of biofilms identifies areas of high pollution. All of the downstream biofilms have high levels of five to eleven drugs (up to 965 ng/g). (Among the 12 analyzed). Additionally, exposure to the discharge point alters the

diversity of the bacterial communities and multiplies the prevalence of resistance integrons (three to 31-fold for Class 1) (for example cyanobacteria). The current research supports that the aquatic environment is impacted by the discharge from WWTPs [5].

The MBBR processes have been widely used in the treatment of municipal and commercial wastewater for BOD/COD removal, as well as for nitrification and denitrification. The city applications are the main topic of this essay. There is a presentation and discussion of the most popular procedure combinations. The presentation includes both fundamental design information gleaned from the study and information derived from actual plant operations. It is shown that the MBBR can be used in a secondary treatment process that is highly compact and high-rate (1 h total HRT). P-removal is necessary for the majority of European plants, and performance information from MBBR and chemical precipitation plants is given. Additionally, information is provided from facilities in Italy and Switzerland that are using nitrification in addition to secondary treatment. Discussion is held regarding the findings from three Norwegian plants that employ the so-called MBBR method for combined denitrification. At low temperatures (11 °C), complete nitrification was proven at nitrification rates as high as 1.2 g NH4-N/m2 d, while denitrification rates reached 3.5 g NO3-Nequiv./m2.d. The overall HRT of the MBBR for N-removal will be in the range of 3 to 5 h, depending on the degree of pretreatment[6].

The use of biofilm as an alternative technology for the treatment of wastewater is covered in this review paper under a variety of loading and operation circumstances. The use of biofilm technology has increased over the past few years as a result of the world's expanding population and the need for clean water supplies. Besides, conventional wastewater treatment plants like activated sludge process present some shortcomings such as not very flexible method (if there is a sudden change in the character of sewage and the effluent of bad quality is obtained), so a better system is urgently needed to provide additional capacity with the least possible cost and to meet the standard effluent by the local authorities. Additional treatment capability is constantly required due to the increased wastewater inflow and organic loading at the treatment facilities. This paper presents fundamental research on biofilm in parts that discuss its application and compare old and new biofilm as well as suspended and fixed film. Additionally, explanations are provided for un-submerged fixed film trickling filter systems and rotating biological contactors. Bed varieties include moving beds, fixed beds, and floating beds. The removal of nitrogen and phosphorus from nutrients and the use of nanotechnology in a biofilm are also described. Discussions also include findings from studies of various applications conducted at the laboratory and pilot scales [7].

We looked into the receiving stream's biogeochemical processing of dissolved inorganic N (DIN) inputs from a wastewater treatment plant's (WWTP) discharge. Along a stream reach downstream of a WWTP, we looked at longitudinal trends of NH4+ and NO3 concentrations and their 15N signatures. To determine the function of stream biofilms in N metabolism, we compared the 15N signatures of epilithic biofilms with those of DIN. To determine whether light limits how well biofilm communities operate, we examined the 15N signatures of the biofilms covering the light- and dark-side surfaces of cobbles separately. In order to determine whether alterations in the environment had an impact on N biogeochemical processes, we collected samples in two different seasons (winter and summer). The research area was capable of transforming and eliminating DIN, but the importance of various biogeochemical pathways for N processing varied according to the season. Downstream N fluxes were affected by nitrification and assimilation during the winter. A substantial difference between the 15N signatures of light- and dark-side biofilms, which suggests that nitrification was mostly associated with dark-side biofilms, revealed that these processes

were spatially segregated at the microhabitat scale. Summertime saw an increase in N processing, and denitrification emerged as a significant N removal route. The light-side and dark-side biofilms had similar 15N signatures, which suggests that N cycling processes are less spatially segregated at this microhabitat size. Overall, our findings demonstrate the ability of streams affected by WWTPs to modify and remove N inputs derived from WWTPs and point to the vital role that biofilms play in these in-stream processes [8].

Biofilms can have a detrimental impact on a variety of industries and sectors, including the biomedical, environmental, and food industries. Biofilm production can be significantly influenced by factors that affect how microbes grow and develop, including temperature, nutrients, and pH, among others. Staphylococcus species are able to endure a broad variety of temperatures, dryness, dehydration, and low water activity in the natural environment. So, our goal was to assess how external environmental variables affected the development of a biofilm of staphylococci that were isolated from hospital wastewater and surface waters. We looked at how methicillin-resistant and methicillin-susceptible S. aureus (MRSA and MSSA) and coagulase-negative staphylococci (CoNS) formed biofilms in different temps, pH levels, salt concentrations, glucose concentrations, and aerobic and anaerobic environments. Compared to MSSA and MRSA, CoNS was able to generate more biofilm biomass. After 24 hours of incubation, all environmental variables that were examined had an impact on the staphylococci isolates' ability to form biofilms. For MSSA and CoNS, higher biofilm development was attained at 4% NaCl and 0.5% glucose, while for MRSA isolates, it was at 1% NaCl and 1.5% glucose. Isolates formed more biofilms at 25 °C and 37 °C than at 10 °C and 4 °C. Compared to pH levels of 9 and 5, pH values between 6 and 8 promoted more robust biofilm development. Even though staphylococci are facultative anaerobes, oxygen increased the likelihood of biofilm development. The findings showed that a variety of environmental variables have an impact on staphylococci biofilm development. The development of MRSA, MSSA, and CoNS strains' biofilms is influenced differentially by various circumstances.

CONCLUSION

Due to the increase in industrial activity around the globe in recent decades, significant amounts of pollutants have been released into the aquatic environment. These pollutants are typically identified by how toxic they are to both living things and the ecosystem. Environmental laws are placing restrictions on a variety of pollutants in industrial wastewater in response to these risks. The scientific community is concerned about the wide variety of production processes and raw materials used by industries as they work to describe effective control technologies. Technologies for biological therapy were thought to be appealing substitutes for traditional approaches. Due to their clear benefits over traditional methods, biofilm-mediated processes for industrial wastewater treatment are in fact among the most effective technologies. The effectiveness of the biofilm mode to increase total pollutant degradation has been discussed. The full-scale use of biofilm wastewater treatment would benefit from increased understanding in the field. This chapter gives a general summary of the useful application of biofilm mode in depollution technologies and engages in a critical analysis of several recent studies that have focused on biofilm-based processes.

REFERENCES:

[1] S. Sehar and I. Naz, “Role of the Biofilms in Wastewater Treatment,” in *Microbial Biofilms - Importance and Applications*, 2016. doi: 10.5772/63499.

[2] H. C. Flemming, “Biofilms and environmental protection,” *Water Sci. Technol.*, 1993, doi: 10.2166/wst.1993.0528.

[3] L. Feng, Z. Wu, and X. Yu, "Quorum sensing in water and wastewater treatment biofilms," *J. Environ. Biol.*, 2013.

[4] Z. Lewandowski and J. P. Boltz, "Biofilms in Water and Wastewater Treatment," in *Treatise on Water Science*, 2011. doi: 10.1016/B978-0-444-53199-5.00095-6.

[5] E. Aubertheau, T. Stalder, L. Mondamert, M. C. Ploy, C. Dagot, and J. Labanowski, "Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance," *Sci. Total Environ.*, 2017, doi: 10.1016/j.scitotenv.2016.11.136.

[6] H. Ødegaard, "Innovations in wastewater treatment: The moving bed biofilm process," *Water Sci. Technol.*, 2006, doi: 10.2166/wst.2006.284.

[7] K. Shahot, A. Idris, R. Omar, and H. M. Yusoff, "Review on biofilm processes for wastewater treatment," *Life Sci. J.*, 2014.

[8] M. Ribot, E. Martí, D. Von Schiller, F. Sabater, H. Daims, and T. J. Battin, "Nitrogen processing and the role of epilithic biofilms downstream of a wastewater treatment plant," *Freshw. Sci.*, 2012, doi: 10.1899/11-161.1.

CHAPTER 7

BIOFILMS CAN GROW NATURAL SURFACES

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ABSTRACT:

Biofilm societies are found everywhere. Every environment with water, nutrients, and a surface is home to them. Biofilms have been discovered all over the world, including the ice-covered arid regions of the Antarctic, the ocean's deep, and the spaces between rocks thousands of feet below the surface of the earth. You name it biofilms can develop in fresh water, salt water, oil pipelines, the human body, etc. Almost any type of locally occurring moisture is acceptable. Bacterial biofilms are present on a variety of body surfaces, including the epidermis, teeth, and mucosa. A biofilm is something that develops on molars and is called plaque. The majority of microorganisms can create biofilms. Both planktonic and biofilm bacteria are susceptible to environmental changes. A mixed culture of bacteria was exposed to unfavorable pH and temperature conditions, which sped up the development of biofilm and increased the electroactivity of the bacteria in microbial electrochemical systems. Quorum sensing is another way that cells can interact, and it may have an impact on biofilm processes like detachment. Although biofilms can contain human infectious organisms in the environment, they can also aid in the cleanup of contaminated soils and groundwater. They aid in the extraction of metals and are crucial to the natural process of recycling waste on Earth.

KEYWORDS:

Bacterial Biofilms, Cells Biofilms, Environmental Factors, Nitrogen-Fixing, QuorumSensing.

INTRODUCTION

Biofilms are a common feature of organic living. Every type of microorganism has a means of adhering to surfaces and other microorganisms. Any non-shedding surface that is in a non-sterile aqueous or humid atmosphere will develop a biofilm. The most extreme habitats can support the growth of biofilms, including frozen mountains and extremely hot, briny hot springs with waters that range in pH from very acidic to very alkaline.

The majority of streams and rivers have rocks and pebbles at their bottoms, and biofilms frequently develop on the top of still pools of water. In waterways and streams, biofilms play a significant role in the food chains because they are grazed by the aquatic invertebrates that many fish eat. Plant surfaces and interiors both contain biofilms. As in the case of nitrogen-fixing rhizobia on root nodules, they can either coexist symbiotically with the plant or add to crop disease. Citrus canker, Pierce's disease of grapes, and bacterial spots in plants like peppers and tomatoes are a few examples of crop illnesses linked to biofilms. Various surfaces have been documented for the growth of biofilms.

One kind of wastewater treatment device is a trickling filter (Figure. 1). Sewage or other wastewater runs downhill over a fixed bed of rocks, coke, gravel, slag, polyurethane foam, sphagnum peat moss, ceramic, or plastic media, which encourages the growth of a layer of microbial slime (biofilm) that covers the bed of media.

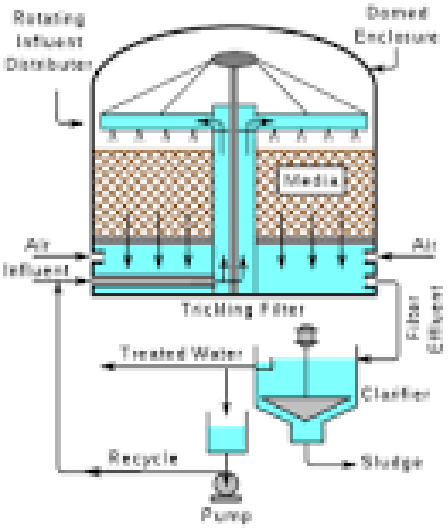

Figure 1: A deceptive filter a diagram illustrating the biofilms' home in a trickling filter (encrypted)

Slow sand filters are employed in the process of water purification to cleanse raw water and create potable water. They function by forming a biofilm in the upper few millimeters of the fine sand layer known as the hypogeal layer or Schmutzdecke. The Schmutzdecke, which is composed of bacteria, fungi, protozoa, rotifera, and a variety of aquatic insect larvae, is produced during the first 10 to 20 days of operation. More algae tend to grow as an epigeal biofilm gets older, and bigger aquatic organisms like some bryozoa, snails, and annelid worms may be present. The underlying sand acts as a support medium for this biological treatment layer, and the top biofilm is the layer that effectively filters potable water. Ppapers of foreign material are caught in the mucilaginous matrix as water travels through the hypogeal layer, and soluble organic material is adsorbed. Microbes, fungi, and protozoa break down the pollutants. An excellent slow sand filter will produce water of excellent quality with a 90–99% decrease in bacterial cell count.

The surfaces of both living and non-living things, as well as abiotic surfaces, are frequently covered in microbial biofilms. Exopolysaccharides and cellular appendages help bacteria in biofilms live in intricate arrangements that, in the end, create a niche where individuals can cooperate and potentially benefit by enduring harsh environmental conditions. It is well known that by enabling bacteria to survive, biofilms in animals can contribute to chronic diseases. The contributions of biofilm formation in the context of rhizobia in symbiotic relationships with plants are still not completely understood. In this chapter, we go over the rhizobia that create biofilms and discuss the implications for plant growth and development [1].

Communities of viruses, bacteria, fungi, and Eukarya make up the gut microbiome (Figure.2), which exists as biofilms. In a healthy state, these biofilms cling to the exterior of the intestinal mucus rather than the epithelium. Invasive pathobionts may be produced from these commensal communities as a result of disturbances to the balance between these biofilms and the host, which could add to the pathogenesis of the disease. When comparing the microbiota of low-income and industrialized nations, environmental variables appear to outweigh genetics in deciding the changes in microbiota populations and function. The

findings covered in this paper have a huge potential for the creation of novel treatments that target the phenotype of microbiota dysbiosis.

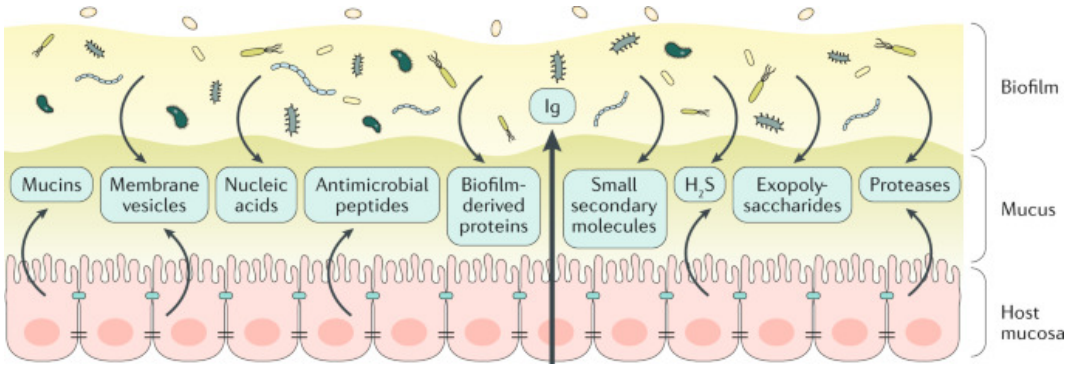

Figure 2. Gut microbe: Diagramed showing the presence of the different microbes in the gut (nature).

The most prevalent and active form of microbial life on Earth is represented by biofilms, which are communities contained within a matrix. Biofilms are extremely relevant to all of the environments they inhabit because they are naturally more productive than any comparable planktonic community. However, the general population, conservationists, and environmental policymakers still have a limited understanding of their existence and significance. The majority of microorganisms in multicellular organisms, such as people, animals, and plants, exist as real biofilms or structures that resemble biofilms and are essential to their growth, physiology, and immunity. On the other hand, some biofilms may harm the health of the recipient.

Many terrestrial and marine environments depend on biofilms growing on non-biological surfaces because they are the foundation of food webs and guarantee nutrient cycling and bioremediation in natural systems (Figure.3). Environmental biofilms, however, have several negative effects on human health as well as the environment, including the promotion of human pathogen survival, the production of toxic byproducts, the contamination of natural and artificial surfaces, and corrosion.

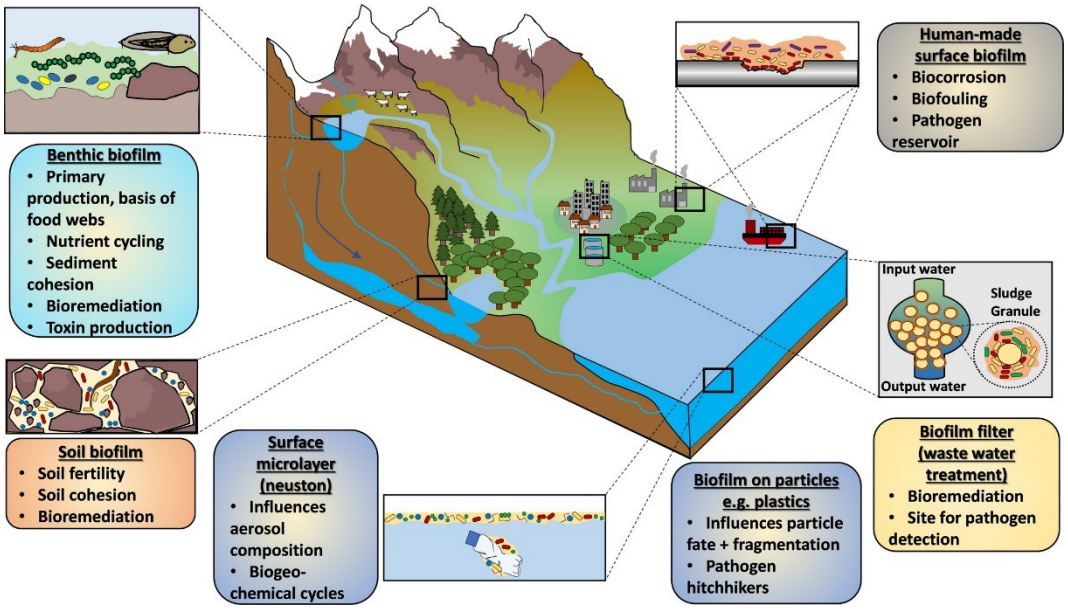

Figure 3. Microbial habitat: Diagramed showing the different habitats of the microbe (british ecology).

The epidemiology of newly emerging infectious diseases in wildlife is poorly understood, even though these diseases pose a danger to public health, biodiversity, and sustainability. The structure and operations of biofilms are impacted by all of the worldwide environmental change drivers. However, little is known about the effects on the welfare of the host and ecosystem. While in medicine and conservation biology the idea of a healthy microbiome (as opposed to dysbiosis) is developing, the idea of a healthy biofilm has not yet been established in environmental studies. Here, use of recent information regarding the functions of biofilms growing on both biological and non-biological surfaces. Understanding the effects of global environmental change on these communities and, in turn, on the health of people, animals, plants, and ecosystems, will be made easier by giving the biofilm life form its due significance.

LITERATURE REVIEW

While plastic pollution is widespread in aquatic ecosystems around the globe and many of its negative effects are well known, it also serves as a novel substrate for a variety of organisms. In aquatic settings, biofilms—assemblages of bacteria, algae, and fungi—colonize hard surfaces. They play an important role in biogeochemical cycling, provide sustenance for grazing organisms that make up a foundational aquatic community and are known to have an impact on how plastic pollution behaves in aquatic environments. In one of the most temporally thorough assessments of biofilm development on freshwater plastics, here we report on the evolution of algal biofilm assemblages on three plastic polymers (Low-Density Polyethylene, Polypropylene, and Polyethylene Terephthalate) over six weeks in the photic and aphotic zones of a freshwater reservoir in Staffordshire, UK. Total algal photosynthetic pigment concentrations did not differ substantially between polymers in either zone, despite significant differences between diatom assemblages quantified on weeks 2, 4, and 6 of the research and those on plastics in the photic and aphotic zones. According to scanning electron microscopy, polymer surfaces degrade within six weeks in the aphotic zone, which could have an impact on plastic disintegration and microplastic production [2].

Similar to how they do in nature, microorganisms inhabit different ecological niches in the human habitat. Human tissue surfaces are colonized by biofilms, which are the main types of multicellular communities. Numerous microorganisms with related but distinct lifestyles live in the gastrointestinal system as isolated planktonic cells, biofilms, and biofilm-dispersed forms. Therefore, taking into account not only the planktonic lifestyle but also biofilms and biofilm-dispersed forms is crucial for understanding homeostatic and changed host-microorganism interactions. In this Review, we cover the biogeographical localization, taxonomic stratification, and trans-kingdom interactions that take place within the biofilm habitat, as well as the natural order of microbes at gastrointestinal surfaces. We also go over the current research theories for biofilms. We evaluate the role of the host-mucosa biofilm interaction in maintaining gut homeostasis and contributing to diseases. The organization, structure, and makeup of mucosal biofilms can be influenced by host variables, and biofilms themselves can play a role in several pathological and homeostatic processes in the gut. Future research on the nature, physical characteristics, composition, and intrinsic communication of biofilms may provide fresh insights into gut physiology and suggest innovative therapeutic choices for gastrointestinal disorders [3].

In comparison to single cells, biofilms are a type of collective life that exhibit emergent properties that benefit its members greatly. They also exhibit a much higher degree of organization. There is, however, no current worldwide analysis of the prevalence of biofilm. In light of the inherent uncertainty, we provide a critical analysis of the term "biofilm" and collect the most recent estimates of the total number of cells in the world's main microbial

habitats. The "big five" habitats—deep continental subsurface (3 1029), deep oceanic subsurface (4 1029), upper oceanic sediment (5 1028), earth (3 1029), and oceans (1 1029)—hold the majority of bacteria and archaea on Earth (1.2 1030 cells). There are orders of magnitude fewer cells in the surviving habitats, which include groundwater, the atmosphere, the ocean surface microlayer, people, animals, and the phyllosphere. Except for the oceans, biofilms rule all habitats on the surface of the Earth, making up about 80% of all bacterial and archaeal cells. We assume that 20–80% of the cells in the deep subsurface exist as biofilms; however, they are not always able to be distinguished from solitary sessile cells in the deep subsurface. Thus, 40–80% of all organisms on Earth are found in biofilms. We conclude that biofilms are the primary mode of active bacterial and archaeal life [4] and that they control all ecological processes.

Communities of bacteria that are enmeshed in a self-produced matrix of extracellular polymeric compounds are what make up bacterial biofilms. (EPS). A collection of "emergent properties" that distinguish bacteria in biofilms from free-living bacterial cells is significant. In this Review, we consider the fundamental role of the biofilm matrix in establishing the emergent properties of biofilms, describing how the characteristic features of biofilms such as social cooperation, resource capture, and enhanced survival of exposure to antimicrobials all rely on the structural and functional properties of the matrix. The ecological success of biofilms as habitat creators and, more broadly, as a bacterial lifestyle [5] is highlighted in our final point. This highlights the importance of an ecological viewpoint in the study of the emergent properties of biofilms.

Bacteria in the Enterobacteriaceae family have characterized surface-associated amyloid fibrils, but it is unclear how much amyloid adhesin is present in natural biofilms. Thioflavin T and two conformationally specific antibodies that target amyloid fibrils were used in this research to specifically stain amyloid adhesins. To connect phenotype with identity, these three distinct detection techniques were each coupled with fluorescence in situ hybridization using fluorescently labeled oligonucleotide probes. As controls, curli mutants of Escherichia coli were used, both with and without amyloid adhesins. Bacteria that produce extracellular amyloid adhesins have been found in biofilms from four distinct natural habitats. These bacteria belong to the phyla Proteobacteria (Alpha-, Beta-, Gamma-, and Deltaproteobacteria), Bacteriodetes, Chloroflexi, and Actinobacteria, among others. According to the habitat, the prokaryotes that produced amyloid adhesins made up between 5 and 40% of all the microbes found in the biofilms. A large amount of amyloid-positive bacteria were discovered, particularly in drinking water biofilms. Environmental isolates from the Gammaproteobacteria, Bacteriodetes, Firmicutes and Actinobacteria verified the production of amyloids. The novel method is a very helpful tool for future culture-independent research in mixed microbial communities, where amyloid adhesin-expressing bacteria appear to be much more abundant and diverse than previously thought[6].

At four surface water supplies in The Netherlands, slow sand filtration combined with thorough pretreatment lowers the microbial growth potential of drinking water to a minimum level. The potential of these slow sand filtrates (SSFs) to promote microbial growth in warm tap water installations was assessed by measuring biofilm formation and growth of Legionella bacteria on glass and chlorinated polyvinylchloride (CPVC) surfaces exposed to SSFs at 37□±□2°C in model system for up to six months. On the glass, the steady-state biofilm concentration varied from 230 to 3,980 pg ATP cm2, while on CPVC, it was 1.4 (0.3) times higher. These concentrations were raised roughly two times by combining cold and heated (70°C) SSFs, and they substantially correlated with the warm water's assimilable organic carbon (AOC) concentrations (8 to 24 g acetate-C equivalents [ac-C eq] liter1).

Legionella pneumophila was able to grow in all biofilms, with maximum concentrations varying from 6 102 to 1.5 105 CFU cm2. After about 50 days of contact, Betaproteobacteriales, primarily Piscinibacter, Caldimonas, Methyloversatilis, and an uncultured Rhodocyclaceae bacterium, predominated in biofilms. These quickly expanding main colonizers most likely served as food for the *L. pneumophila* host amoebae. 37.5% of the clones recovered were alphaproteobacteria, mostly Xanthobacteraceae, such as Bradyrhizobium, Pseudorhodoplanes, and other amoeba-resistant bacteria. The variations in the Legionella CFU pg1 ATP ratios in the biofilms are explained by a conceptual model based on a quadratic connection between the *L. pneumophila* colony count and the biofilm concentration under steady-state conditions [7].

CONCLUSION

All ecosystem strata contain bacteria, and it has recently been discovered that all microbes have the ability to create biofilms. Extracellular polymeric substances, also known as biofilm, are a collection of microorganisms that are condensed inside a polysaccharide matrix and attached to a solid support. The reliability and growth of biofilms are demonstrated by the various interactions that the various microorganisms in the biofilms have with one another, including horizontal gene transfer. Aquatic environments frequently contain biofilms, which promote corrosion, control biogeochemical cycles, invertebrate larval settlement, bacterial communication, and many other processes. Because they are found in environments with severe environmental conditions, biofilms are also well known for their extraordinary resistance to a variety of physical and chemical stresses, including pH, salinity, pressure, and antibiotics.

It is also known that a variety of human pathogens can create biofilms, which can be fatal in situations where the immune system is compromised. Due to the continuous release of cells from biofilms and resistance to antibiotics, microbial biofilms connected to ophthalmology, implanted catheters, chronic wounds, and dental plaques exhibit bacterial infections that last for a long time. Biofilms also explained how their associated microbes colonized the surfaces of animals and plants. Recent studies have indicated that certain bacteriophages, as well as phage susceptibility and resistant bacterial phenotypes within the biofilms, promote biofilm assembly and function. This chapter will highlight and expand on the nature of biofilms in various environments.

REFERENCES:

[1] A. A. Angus and A. M. Hirsch, “Biofilm Formation in the Rhizosphere: Multispecies Interactions and Implications for Plant Growth,” in *Molecular Microbial Ecology of the Rhizosphere: Volume 1*, 2013. doi: 10.1002/9781118297674.ch66.

[2] I. L. Smith, T. Stanton, and A. Law, “Plastic habitats: Algal biofilms on photic and aphotic plastics,” *J. Hazard. Mater. Lett.*, 2021, doi: 10.1016/j.hazl.2021.100038.

[3] J. P. Motta, J. L. Wallace, A. G. Buret, C. Deraison, and N. Vergnolle, “Gastrointestinal biofilms in health and disease,” *Nature Reviews Gastroenterology and Hepatology*. 2021. doi: 10.1038/s41575-020-00397-y.

[4] H. C. Flemming and S. Wuertz, “Bacteria and archaea on Earth and their abundance in biofilms,” *Nat. Rev. Microbiol.*, 2019, doi: 10.1038/s41579-019-0158-9.

[5] H. C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice, and S. Kjelleberg, “Biofilms: An emergent form of bacterial life,” *Nature Reviews Microbiology*. 2016. doi: 10.1038/nrmicro.2016.94.

[6] P. Larsen, J. L. Nielsen, M. S. Dueholm, R. Wetzel, D. Otzen, and P. H. Nielsen, "Amyloid adhesins are abundant in natural biofilms," *Environ. Microbiol.*, 2007, doi: 10.1111/j.1462-2920.2007.01418.x.

[7] D. van der Kooij, H. R. Veenendaal, R. Italiaander, E. J. van der Mark, and M. Dignum, "Primary colonizing Betaproteobacteriales play a key role in the growth of Legionella pneumophila in biofilms on surfaces exposed to drinking water treated by slow sand filtration," *Appl. Environ. Microbiol.*, 2018, doi: 10.1128/AEM.01732-18.

CHAPTER 8

APPLICATION OF BIOFILMS TO MARINE CULTURE

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ABSTRACT:

A biofilm is a collection of microbial cells that have become permanently attached to a surface and are contained in a polysaccharide-based framework. It can develop on a broad range of surfaces, such as living tissues, medical equipment, pipes in industrial or potable water systems, and aquatic ecosystems in nature. The biofilm assemblage may contain well-diversified creatures, such as bacteria, arthropods, algae, protozoa, and protozoa. The hydrodynamics of the system, the abundance of nutrients, the amount of light, and the ability of the organism to graze are all factors that affect the biofilm's structure. According to observations, the advent of substrates for the growth of biofilm in the aquaculture system is crucial. Microorganisms that form biofilms are very nutrient-rich and tiny. In contrast to planktonic organisms in the water column, the organisms of biofilm can function as single-cell proteins and are simple for all-size cultured species in aquaculture to harvest. Biofilms are regarded as an excellent source of protein (23–30%). Microalgae and heterotrophic bacteria are abundant sources of bioactive compounds, dietary stimulants, and immune boosters that can improve the development efficiency of cultured organisms. By giving cultured organisms a spot to hide and find shelter, substrates reduce mortality. Through the nitrification process, the attached nitrifying bacteria in biofilm enhance water purity by reducing ammonia waste from culture systems. Low-cost technology based on biofilms will assist resource-strapped farmers in producing protein-rich nutrients from aquaculture in a sustainable way. The purpose of this study is to discuss the function of biofilm in aquaculture.

KEYWORDS:

Aquaculture Biofilms, Biofilms Aquaculture, Bacterial Biofilm, Bacterial Cells, Planktonic Organisms.

INTRODUCTION

A biofilm is a complicated community of autotrophic and heterotrophic organisms, such as bacteria, protozoans, fungi, and algae, embedded in an extracellular polysaccharide matrix that is secreted by the bacteria (Figure. 1). Biofilms are organic layers that form on substrates with submerged surfaces. The first step in the creation of heterotrophic food is the biofilm formation of microbes on the substrates. In addition to being directly used by fish, this microbial biofilm has a great deal of potential to sustain organisms that are fish food. Much planktonic fish, including silver carp, rohu, catla, mullets, and milkfish, can collect biofilm of the microbial community in blocks of 20–60 u in water and sediment. The organic and mineral fractions of organic manure serve as an abundant supply of nutrients and energy for the microbial population. These organisms can be directly harvested by fish in large numbers. The detritus's comparatively indigestible substrate and microbial film coating are both digested, but the substrate itself travels through the fish gut relatively undamaged and is later recolonized by microbes and harvested by fish.

The controlled cultivation of aquatic creatures such as fish, crustaceans, mollusks, algae, and other valuable organisms like aquatic vegetation is known as aquaculture (less frequently spelled as aquiculture[1]. (e.g. lotus). Contrasted with industrial fishing, which involves capturing wild fish, aquaculture involves raising populations of freshwater, brackish water, and saltwater species in controlled or semi-natural environments [2]. Mariculture, also known as marine farming, is the term used to describe aquaculture that is carried out in lagoons and other seawater environments as opposed to freshwater habitats. Pisciculture is a subset of aquaculture that involves raising fish for culinary purposes.

The breeding, cultivation, and harvesting of fish and other aquatic vegetation are referred to as aquaculture, also known as farming in water. It is a sustainable food supply and industrial product that helps restore populations of aquatic species that are in danger of extinction and creates healthier habitats (Figure.1). Due to the rising demand for seafood, technology has boosted fish growth in open oceans and coastal marine waterways [3].

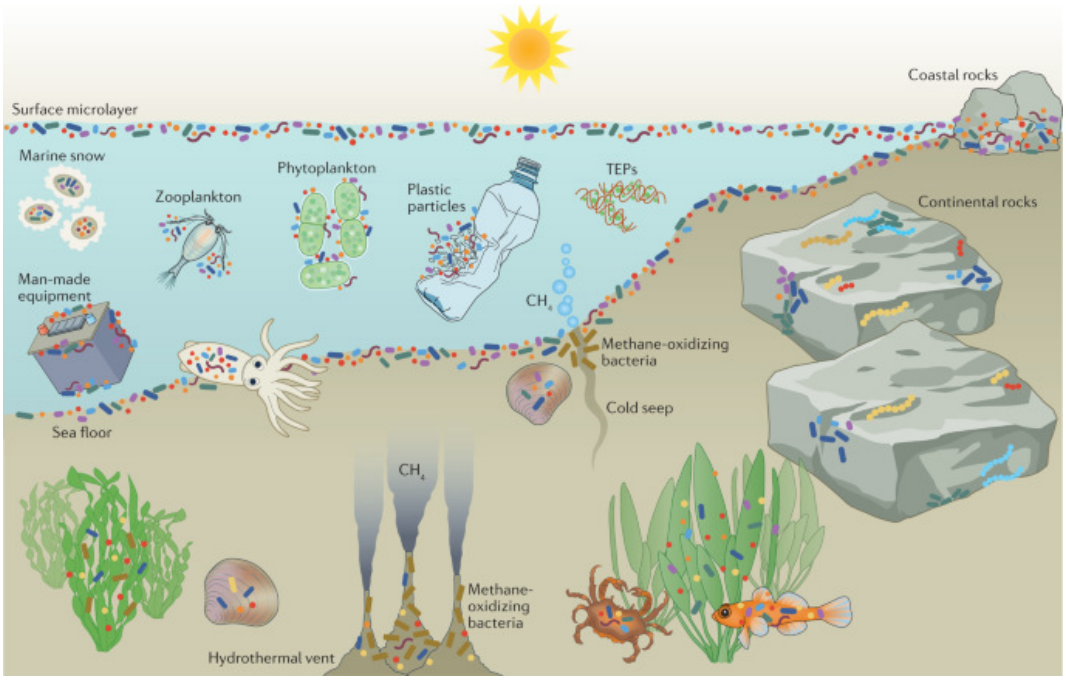

Figure 1: Marine diversity: Diagramed showing the biodiversity of the marine system (Nature).

As in the case of fish tanks, ponds, aquaponics, or raceways, aquaculture can be practiced in entirely artificial facilities that are created on land (onshore aquaculture), where the living circumstances, such as water quality (oxygen), feed, and temperature, are under human control. Alternatively, they can be conducted on well-sheltered shallow waters nearshore of a body of water (inshore aquaculture), where the cultivated species are subjected to a relatively more naturalistic environment; or on fenced/enclosed sections of open water away from the shore (offshore aquaculture), where the species are either cultured in cages, racks or bags and are exposed to more diverse natural conditions such as water currents (such as ocean currents), diel vertical migration and nutrient cycles.

Aquaculture pools can benefit from the use of biofilms, and understanding the microbial succession process in biofilms would help characterize metabolic processes and enable optimization. In the current research, high-throughput sequencing was used to look into the microbial succession of a biofilm growing on the artificial substrate in a subtropical freshwater pond. Artificial substrata successfully decreased the pond's total nitrogen and

phosphorus concentrations. The Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria phyla were the most prevalent in the adult biofilm. There was a substantial increase in the relative abundance of denitrifiers and phosphorus-removing bacteria, like those found in the Comamonadaceae and Neisseriaceae[2].

Although biofilms are frequently viewed negatively, their traits can occasionally be used to one's advantage. Their ability to trap organic nutrients is effectively applied in wastewater treatment to lower organic content before release. For biological nitrification or denitrification of wastewater, biofilms are the favored method. The autotrophic microorganisms Nitrosomonas and Nitrobacter carry out nitrification in two steps. Any aerobic biological treatment method, including the suspended growth, activated sludge, attached (supported), trickling filter, or packed bed processes, can accomplish it. It is also possible to accomplish nitrification by using rotating biological contactors. Many aquaculture production methods for recycling using rotating biological contactors. It would be reasonable to anticipate that the nitrifying and nonnitrifying biofilms in these environments would have different attachment characteristics. Temperature, substrate concentration in the bulk liquid, and disc spinning rates all affect biofilm thickness in rotating contactors.

Some Pseudoalteromonas sp. from marine habitats can inhibit *Vibrio anguillarum* (*V. anguillarum*) in a live cell assay; they produce bioactive secondary metabolites and this production of secondary metabolites, which comprises small molecules, antibiotics, and pigments, takes place during the stationary phase when bacterial physiology changes. Additionally, secondary metabolites may function as signaling molecules to shield biofilms from microbial invasion. The stationary period of bacterial growth is represented by biofilm formation in natural environments. Fish spawn (deposits on this specially prepared abiotic surface) is thought to be protected from fish pathogenic bacterial and/or fungal infestation by Pseudoalteromonas sp. biofilm development. Aquaculture and ornamental fish husbandry may benefit from using Pseudoalteromonas sp. for larvae rearing[3].

Both planktonic and biofilm bacteria are susceptible to environmental changes. A mixed culture of bacteria was exposed to unfavorable pH and temperature conditions, which sped up the development of biofilm and increased the electroactivity of the bacteria in microbial electrochemical systems. One of the most extreme forms of stress an organism can experience is nutrient deprivation. One easy but powerful way to encourage the development of biofilms is to restrict the supply of nutrients. Bacterial cells adhering to a surface is the first stage in the formation of a biofilm. Staphylococcus biofilm development is significantly impacted by surface chemistry, according to studies using various microtiter plate materials. Biofilm development can be influenced by chemically altering a surface's hydrophobicity and surface charge by adding or removing particular functional groups.

Shewanella oneidensis MR-1 biofilms were made to adhere to the carbon-felt anode of a microbial fuel cell through treatment with UV light and ozone gas, which led to a rise in the current generation. In their study, Sarjit et al. detailed various surface modifications that have been applied to improve biofilm formation in particular bioelectrochemical system applications like fermentation, bioremediation, biosensing, and energy recovery. By creating novel, inexpensive, biocompatible materials that make it easier for bacterial cells to adhere to surfaces and form biofilms, there is potential to improve the effectiveness of these applications. The performance of electroactive biofilms and bacterial colonization may both be favorably impacted by altering the surface topography on a microscale or lower.

Bacterial cells begin to aggregate after adhering to the surface and encase themselves in a self-produced matrix of extracellular polymeric materials. Long-chain polyunsaturated fatty

acids greatly increased biofilm formation in *Acinetobacter baumannii* and *Klebsiella pneumoniae(Staphylococcus aureus*, *Listeria monocytogenes*, and *Streptococcus mutans* were all able to produce more EPS when exposed to simple carbs like glucose, glucosamine, and N-acetylglucosamine. Divalent cations, such as Ca2+ and Mg2+, when added, help Pseudomonas spp. create biofilms by creating electrostatically mediated cross-links in the matrix that keep the bacterial cells cohesive. Salt stress has been shown to cause enhanced cell adhesion to surfaces in studies on Gram-negative bacteria like *Shigella boydii* and *Salmonella enterica,serovar Typhimurium*. These findings suggest that there are numerous opportunities for introducing interventions to enhance biofilm formation in the physical processes related to bacterial cell attachment and aggregation.

LITERATURE REVIEW

Less fish biomass is generated in intensive aquaculture techniques compared to the feed that is consumed. The aquatic environment is harmed by the aquaculture system's wastewater discharges. To control waste nutrient generation and decrease the use of fish meals as feed, it is necessary to develop efficient technology. It was discovered that biofilm-based aquaculture is a promising technology for extensive aquaculture practices that lower the use of feed and the generation of waste nutrients. Additionally, biofilm serves as a good source of protein, a place for shrimp to live while they are molting, and an immunostimulant, and it increases fish output, yield, survival, and health. It also efficiently converts waste nitrogen into high-quality fish and shrimp feed. Recent research has shown that adding substrate and using the right C: N ratio can accelerate fish and shrimp development, enabling biofilm-based aquaculture for intensive culture techniques. It has been discovered that biofilm plays a significant part in making intensive aquaculture practices more sustainable, from nursery rearing to post-stocking management of table-size fish [1].

All parts of an aquaculture system are capable of forming biofilms that contain different aquatic microflora. The pathogenic bacteria that are released from the biofilms have the ability to lead to chronic illnesses. Eight different kinds of materials were examined in seven recirculating freshwater facilities and two recirculating saltwater facilities. Using conventional bacteriological techniques and market kits, pathogenic bacteria were located. Shigella spp., Vibrio spp., and Bacillus cereus were the three main human diseases. Aeromonas hydrophila, Photobacterium damsel, and *Vibrio spp*. were the main pathogens that should be of worry for fish. Not between building materials, but between facilities, was the most notable variation in the occurrence of biofilm pathogens. According to this research, biofilms in recirculating aquaculture systems may serve as another reservoir for pathogenic bacteria [4].

This study's goal was to find out if typical bacterial catfish pathogens could adhere to and colonize surfaces that are present in aquaculture plants. We also looked at calcium's part in biofilm development. Five bacterial pathogens were attached to polystyrene dishes, and the amount of biofilm they produced was measured. (i.e., *Flavobacterium columnare*, *Aeromonas hydrophila*, *Edwardsiella ictaluri, E. tarda*, and *E. piscicida*). Calcium addition improved the formation of thick biofilms by *Flavobacterium columnare* and *A. hydrophila*. All of the tested Edwardsiella species produced considerably less biofilm, and calcium had little to no impact on this process. A conventional plate count technique was used to measure attachment to both natural and artificial surfaces. The presence of biofilms on the surfaces was confirmed by scanning electron microscopy (SEM). On the liner, flexible PVC, and nets, a biofilm of *Flavobacterium columnare* was produced. Bamboo stopped cell growth and *F. columnare* attachment. On all tested materials, *Aeromonas hydrophila* and *E. ictaluri* formed biofilm, though there were noticeable variations between substrates. *E. ictaluri* was able to

colonize and grow on all of the aquaculture materials tested, but it was unable to create a biofilm on microtiter polystyrene plates. Our findings showed that widespread bacterial diseases could potentially colonize surfaces and could potentially use fish farm biofilms as reservoirs [5].

Antimicrobials, including those crucial to human treatments, are being used more frequently as prophylactic and therapeutic measures as aquaculture expands globally. Approximately 80% of antimicrobials used in aquaculture enter the environment with their activity intact where they select for bacteria whose resistance arises from mutations or more importantly, from mobile genetic elements containing multiple resistance determinants transmissible to other bacteria. Such selection modifies the typical fish and shellfish flora as well as aquatic habitats' biodiversity. The commonality of the mobilome (the total of all mobile genetic elements in a genome) between aquatic and terrestrial bacteria together with the presence of residual antimicrobials, biofilms, and high concentrations of bacteriophages where the aquatic environment may also be contaminated with pathogens of human and animal origin can stimulate the exchange of genetic information between aquatic and terrestrial bacteria. Aquatic bacteria, fish pathogens, and human pathogens all share several newly discovered genetic components and resistance determinants for quinolones, tetracyclines, and -lactamases that appear to have originated in aquatic bacteria. Thus, excessive antimicrobial use in aquaculture has the potential to be harmful to both human and animal health as well as the aquatic ecosystem, and it needs to be better evaluated and regulated[6].

One of the major obstacles to shrimp farming around the world is the presence of infectious diseases in shrimp. Drug therapy is a hot topic right now due to the alarming rise in bacterial drug resistance. Therefore, it is necessary to discourage the use of antibiotic treatment in aquaculture and identify suitable substitutes. Nanoppapers, biofilm-based vaccines, algal extracts, phytobiotics, probiotics, prebiotics, and synbiotics are just a few of the innovative and successful treatments that the fields of nanotechnology and biotechnology have suggested in recent years to fight infectious illnesses. Because they are biologically derived, algal extracts, phytobiotics, probiotics, prebiotics, and synbiotics are acceptable to use in aquaculture environments. Nanomaterials are also becoming a safer option to antibiotic therapy with the development of green and bio-nanotechnology.

Regular immunizations created from antigens of planktonic forms are less effective than those created from antigenic components of bacterial biofilms. Some of these techniques have wide-ranging uses in shrimp aquaculture as immunomodulators, diagnostic instruments, drug and vaccine carriers, and other things. By using these novel techniques in place of antibiotics and other chemical agents, chemotherapy's risks in shrimp aquaculture can be avoided. By implementing these tactics, aquaculture-based food becomes more healthy, and consumer-friendly, and contributes to the development of sustainable aquaculture. This review sheds light on the benefits and knowledge gaps in these strategies that need to be filled, as well as the negative impacts of antibiotic therapy in shrimp aquaculture [7].

Ulva lactuca L., Undaria pinnatifida Suringar, and a trickling biofilm filter were added to systems housing Haliotis iris Gmelin to evaluate the effectiveness of seaweeds and bacterial biofilm for removing nitrogenous wastes from recirculating marine aquaculture. The 14-day experiments were performed in triplicate. Although nitrate levels rose linearly over time, hitting 2.30 mg l1, biofilm filtration kept ammonium levels low (around 0.10 mg l1). Ammonium was reliably kept in seaweeds at lower levels (around 0.03 mg l 1) than those seen with biofilm filtration. Additionally, pH was less variable and nitrates were undetectable, and useful seaweed biomass with increases of up to 50% was produced. Therefore, seaweed filtration has the ability to increase the productivity and efficiency of

recirculating aquaculture through improved culture conditions and the creation of biomass that is commercially valuable[8].

It is suggested that nitrate be removed using a straightforward procedure for use in farming. Pellets made of biodegradable polymer serve as both a biofilm carrier and a firm substrate for denitrification. The viability and a preliminary assessment of the process performance in a recirculated aquaculture system were investigated in laboratory trials using conventional aquaria and fish. The fish were in good shape the entire test time. In comparison to the untreated reference system, the treated aquaria had minimal nitrate concentrations. Another benefit was the stability of pH in denitrification units, whereas the pH of untreated water dropped as a result of nitrification [9].

In aquaculture, where they occur naturally and can be artificially added, microorganisms play a variety of functions. In addition to recycling nutrients and breaking down organic debris, they occasionally infect and kill fish, fish larvae, and live feed. Additionally, some microbes may defend fish and embryos from illness. Monitoring and modifying the microbial communities in aquaculture environments has therefore tremendous potential for evaluating and improving water quality as well as for limiting the spread of microbial infections. Using microbial communities to effectively perform ecosystem services and monitor water quality within aquaculture systems might be possible in a few years. However, before we can effectively manipulate and engineer these microbiomes, we must first completely comprehend the microbiomes of both healthy and diseased aquaculture systems. Similarly to this, by modifying the microbiome, or by using probiotic microorganisms, we can lessen the need to use antibiotics in aquaculture. Recent research has shown that probiotic bacteria can greatly lower the mortality of infected fish larvae and can control fish pathogenic bacteria in the live feed. However, our limited understanding of pertinent microbial interactions and the system's overall ecology presently makes it difficult to manage the aquaculture microbiota effectively [10].

CONCLUSION

A biofilm is a collection of microbial cells that have become permanently attached to a surface and are contained in a polysaccharide-based framework. It can develop on a broad range of surfaces, such as living tissues, medical equipment, pipes in industrial or potable water systems, and aquatic ecosystems in nature. The biofilm assemblage may contain well-diversified creatures, such as bacteria, arthropods, algae, protozoa, and protozoa. The hydrodynamics of the system, the abundance of nutrients, the amount of light, and the ability of the organism to graze are all factors that affect the biofilm's structure. According to observations, the advent of substrates for the growth of biofilm in the aquaculture system is crucial. Microorganisms that form biofilms are very nutrient-rich and tiny. In contrast to planktonic organisms in the water column, the organisms of biofilm can function as single-cell proteins and are simple for all-size cultured species in aquaculture to harvest. Biofilms are regarded as an excellent source of protein (23–30%). Microalgae and heterotrophic bacteria are abundant sources of bioactive compounds, dietary stimulants, and immune boosters that can improve the development efficiency of cultured organisms. By giving cultured organisms a spot to hide and find shelter, substrates reduce mortality. Through the nitrification process, the attached nitrifying bacteria in biofilm enhance water purity by reducing ammonia waste from culture systems. Low-cost technology based on biofilms will assist resource-strapped farmers in producing protein-rich nutrients from aquaculture in a sustainable way. The purpose of this study is to examine the function of biofilm in aquaculture.

REFERENCES:

[1] K. P. P., B. Vivekanand, and K. Kundan, "Biofilm in aquaculture production," *African J. Microbiol. Res.*, 2014, doi: 10.5897/ajmr2013.6445.

[2] Z. Li *et al.*, "Microbial succession in biofilms growing on artificial substratum in subtropical freshwater aquaculture ponds," *FEMS Microbiology Letters*. 2017. doi: 10.1093/femsle/fnx017.

[3] W. Wesseling, "Beneficial biofilms in marine aquaculture? Linking points of biofilm formation mechanisms in Pseudomonas aeruginosa and Pseudoalteromonas species," *AIMS Bioeng.*, 2015, doi: 10.3934/bioeng.2015.3.104.

[4] R. K. King, G. J. Flick, M. D. Pierson, S. A. Smith, G. D. Boardman, and C. W. Coale, "Identification of bacterial pathogens in biofilms of recirculating aquaculture systems," in *Journal of Aquatic Food Product Technology*, 2004. doi: 10.1300/J030v13n01_11.

[5] W. Cai and C. R. Arias, "Biofilm formation on aquaculture substrates by selected bacterial fish pathogens," *J. Aquat. Anim. Health*, 2017, doi: 10.1080/08997659.2017.1290711.

[6] F. C. Cabello *et al.*, "Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health," *Environ. Microbiol.*, 2013, doi: 10.1111/1462-2920.12134.

[7] P. S. Seethalakshmi, R. Rajeev, G. S. Kiran, and J. Selvin, "Shrimp disease management for sustainable aquaculture: innovations from nanotechnology and biotechnology," *Aquaculture International*. 2021. doi: 10.1007/s10499-021-00698-2.

[8] P. L. Cahill, C. L. Hurd, and M. Lokman, "Keeping the water clean - Seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture," *Aquaculture*, 2010, doi: 10.1016/j.aquaculture.2010.05.032.

[9] A. Boley, W. R. Müller, and G. Haider, "Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems," in *Aquacultural Engineering*, 2000. doi: 10.1016/S0144-8609(00)00033-9.

[10] M. Bentzon-Tilia, E. C. Sonnenschein, and L. Gram, "Monitoring and managing microbes in aquaculture – Towards a sustainable industry," *Microb. Biotechnol.*, 2016, doi: 10.1111/1751-7915.12392.

CHAPTER 9

BIOFILMS ARE PROTECTORS FOR THE PLANT GROWTH

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ABSTRACT:

A biofilm is a group of microorganisms that are adherent to a surface permanently and are enclosed in an extracellular polymeric substance (EPS) matrix. By providing protection as described above and increasing the potential of the bacteria to survive and develop in the plant environment, biofilms provide survival sites for both beneficial and opportunistic pathogenic bacteria. In recent years, there has been a growing body of research on the significance of bacterial surface elements in conjunction with functional signals. On or in their stems, leaves, roots, transport organs, and other parts, plants sustain a wide variety of bacteria. The health and output of plants are significantly impacted by these plant-associated bacteria. Symbiotic and pathogenic responses are linked to biofilm formation in plants, but it is unknown how plants control these relationships. It has been discovered that some bacteria in biofilm matrices promote plant development and defend plants against phytopathogens (a process known as biocontrol), whereas other bacteria are involved in pathogenesis. The connection between plants and microbes was covered in this chapter.

KEYWORDS:

Bacterial Biofilms, Bulk Soil, Biological Control, Microbial Community, Plant Growth.

INTRODUCTION

Because some bacteria, fungi, viruses, and protozoa are parasites or pathogens of insects or other organisms that are pests or cause disease in plants, they can be used to safeguard plants. These microorganisms have been utilized for years in the biological management of pests and plant diseases due to their biological characteristics, including in the EU. Microorganisms are found naturally in the environment, and the strains with the best qualities are those that are used in biological control to safeguard crops from pests and diseases. However, before microorganisms can be used, it must be confirmed that their use is secure and has no adverse effects on the health of humans, animals, or other non-target organisms. To combat the deterioration of soil health brought on by the careless application of chemical inputs and loss of soil microbial diversity, widespread adoption of sustainable farming practices is urgently required. By producing enough high-quality food in sufficient quantities, reducing waste and environmental impacts, and making profitable use of nonrenewable resources, such practices help to guarantee an integrated system of crop and livestock production over the long run. This eco-friendly strategy has lately received a lot of attention. Ecosystems can be preserved, agricultural economic stability is promoted, and farmer quality of life is enhanced by the use of sustainable agriculture. Due to these factors, sustainable agriculture must emphasize managing scarce resources while striking a balance between societal, economic, and ecological objectives.

In addition to being a vital instrument for crop production, the soil is also a sophisticated living environment that needs to be viewed holistically. The health of plants, animals, and people is maintained by soil, which also promotes the quality of the air and water environments. To guarantee long-term productivity and stability, healthy soil must be

preserved and protected. As living elements of soil, microorganisms play a crucial role in nutrient cycling, the breakdown of organic matter, and the preservation of soil structure. They are therefore frequently referred to as "natural soil engineers" and can serve as an environmentally friendly substitute to uphold soil health and increase crop output. Due to their capacity to sustain a nutrient-rich soil environment, enhance abiotic stress tolerance, and serve as antagonists against various pathogens, PGPB can be formulated as biobased organic biofertilizers and biopesticides. Additionally, the use of PGPB in agriculture guarantees food safety, has no detrimental effects on the ecosystem, and promotes sustainable crop production.

The rhizosphere can be divided into three zones: the endo rhizosphere is the interior of the root, the rhizoplane is the surface of the root, and the ectorhizosphere is the zone that extends from the rhizoplane to the bulk soil and consists of soil that adheres to the root, in addition to the volume of soil that is not part of the rhizosphere and is not influenced by the root is referred to as bulk soil. Through various plant-microbe interactions and the formation of associations on root and shoot surfaces as complicated, interactive microbial communities, plant growth-promoting bacteria can colonize plants. Numerous ecological processes in the soil, such as nutrient transformation and fixation, organic matter decomposition, and stress reduction, can benefit greatly from the presence of these helpful microbes.

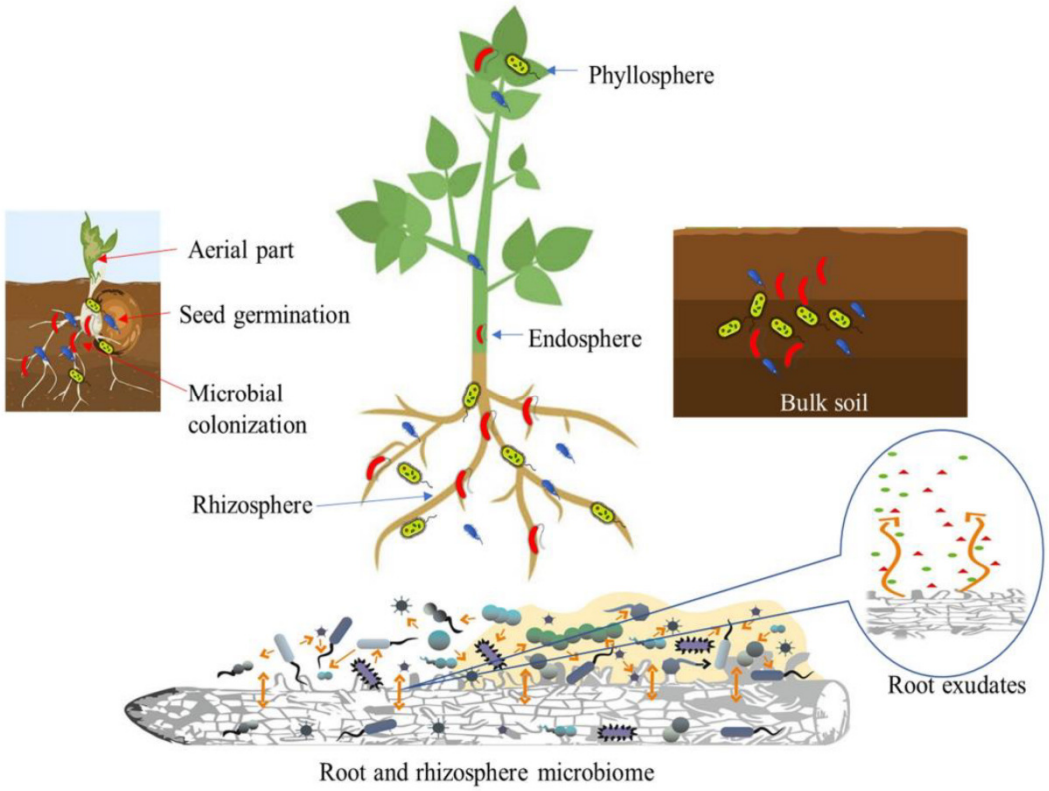

Figure 1: Interaction of the plant and microbes: Diagram showing the plant and microbes interaction region involved in the biofilm formation (MDPI).

The microbial population of soil in the rhizosphere, which surrounds plant roots, is 100–1000 times greater than that of the surrounding soil and is affected by substances secreted by the plant roots. Interactions between PGPB and other taxa in the rhizospheric area include fungi, protozoa, nematodes, and plant viruses. PGPBs are crucial in controlling soil fertility, cycling nutrients, and fostering plant development. The surface and apoplast of leaf tissue (phyllosphere), the rhizosphere, the inner sections of plant tissue (endosphere), and the bulk soil are the main areas where plant-microbe interactions take place. The first organisms to

inhabit plants are those that come from the seed. Rhizosphere microbes that enter the plant through the roots ultimately supplement and partly replace this microbiota that originates from the seed. The complexity of relationships between plants and microbes can differ. The existence of a microbe and its metabolites triggers a response in the plant, and vice versa, the microbe is impacted by the plant's environment and responds to its metabolism and physiology. Exudates from plants draw soil microorganisms in that area toward the root zone. The activities of the microbiota in the root zone, in turn, have a significant effect on plant growth and health (Figure .1). Rhizobia are one type of helpful soil bacterium that colonizes roots and forms microcolonies or biofilms. We recently compiled information on rhizobacteria's ability to attach to surfaces and/or create biofilms. Through several mechanisms, such as the obstruction of xylem vessels, increased resistance to plant antimicrobial substances, and/or enhanced colonization of particular habitats, biofilm development also adds to the virulence of phytopathogenic bacteria. The formation of biofilms and autoaggregation is crucial for bacterial survival and host plant colonization. Bacterial adhesion, cell-cell interactions, plant colonization, and eventually plant-bacterial interactions generally are influenced by a variety of environmental, genetic, and structural factors. In this paper, we review current research on the mechanisms by which bacteria attach to, aggregate into, and form biofilms on plant surfaces.

The idea of biofilm in plant pathogenic fungi presents a chance to take advantage of fresh, ecologically friendly agricultural methods. It is reasonable to expect that interfering with the key steps that orchestrate the genesis of virtually every biofilm (e.g., attachment, cell-to-cell communication, dispersion) could provide a way for new preventive strategies that do not necessarily exert lethal effects on cells, but rather sabotage the propensity for a biofilm lifestyle. These compounds shouldn't exert a selective pressure that would lead to the development of resistance because they don't work by causing the cells to die. Zosteric acid (ZA), a secondary metabolite from the seagrass Zostera marina, reduces fungal adhesion and has a significant impact on the thickness and morphology of fungal biofilms at sub-lethal amounts. Although they are unable to develop filamentous formations, the cells are still metabolically active. Additionally, ZA increases the effectiveness of antibiotics, exhibits cytocompatibility with both soft and hard tissues, has a minimal bioaccumulation potential and has no negative effects on Daphnia magna. By interacting with the NADH: quinone reductase (WrbA), a member of the family of flavoprotein quinone reductases that is extensively present in fungi, ZA affects the balance of oxidative reactions.

The importance of ROS in fungal development and the pathogenicity of several phytopathogenic fungi demonstrate how promising the involvement of ZA in oxidative stress response is as an alternative to traditional control methods. Caripyrin, a newly discovered pyridyloxirane, inhibits conidial germination and appressorium development in P. oryzae without being cytotoxic, antibacterial, or nematicidal.

It was isolated from submerged cultures of the basidiomycete *Gymnopus montagnei* (syn. Caripia montagnei). *Boesenbergia pandurata* (finger root) oil was used at sub-MIC levels, which decreased the development of Candida biofilm by 63-98%. When used at a sub-lethal concentration, purpurin, a naturally occurring red anthraquinone pigment frequently found in madder root, prevented the *C. albicans* yeast-to-hypha transition by suppressing the expression of hypha-specific genes and the hyphal regulator RAS1 [1].

This study emphasizes the significance of biofilm formation as a crucial aspect of interactions between plants and PGPB. Understanding the procedure, mechanisms, and elements affecting PGPB biofilm formation will open up new possibilities for use in the future.

LITERATURE REVIEW

Diseases and microbial changes cause agricultural yields to drop by close to one-third annually. The use of a variety of chemicals that are active against spoilage and unwanted pathogenic microorganisms is the primary control approach to reduce these losses. Their widespread use has caused serious environmental damage, human toxicity, and several illnesses. The use of microbial biocontrol agents is a newly developed alternative to this chemical strategy. Biopesticides have been used successfully in a number of areas, but their mode of action needs to be better understood in order to be better controlled and for their use to be increased. Biofilms are the favored mode of life for microorganisms in the target agricultural biotopes, according to a very small number of studies. Growing data suggests that significant bioprotection mechanisms may be driven by the spatial organization of microbial communities on crop surfaces. The purpose of this review is to provide an overview of the data supporting biofilm formation by biocontrol agents on crops and to explore how this surface-associated mode of life may affect their biology, interactions with other microorganisms and the host, and, ultimately, their overall beneficial activity [2].

Some soil bacteria that coexist with plant roots and can shield their host from pathogenic microorganisms are being developed as biological plant disease control agents. These bacteria's ability to defend plants has been linked to their capacity to secrete a variety of antibiotic substances when grown in isolated cultures under controlled laboratory circumstances as planktonic cells. It is still unclear how these antibiotics are expressed in situ in the rhizosphere, where bacterial cells ordinarily infiltrate root tissues. In this study, we investigated spatiotemporal alterations in the secreted antibiome of *Bacillus amyloliquefaciens* forming as biofilms on roots using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI). Nonribosomal lipopeptides like the plant immunity inducer surfactin or the extremely fungi toxic iturins and fengycins were easily generated in the surrounding medium, albeit in varying times and amounts. Interestingly, we were also able to recognize a novel variant of surfactins released at later time points thanks to tandem mass spectrometry (MS/MS) experiments carried out directly from the gelified culture medium. However, no additional bioactive substances, such as polyketides, were found at any point in time, strongly indicating that the antibiome expressed in planta by *B. amyloliquefaciens* does not represent the extensive genetic arsenal dedicated to the synthesis of such substances. This initial dynamic study demonstrates the effectiveness of MALDI MSI as a tool for locating and mapping antibiotics produced by bacteria associated with roots and, more broadly, for examining molecular plant-microbe interactions[3].

Plant growth-promoting rhizobacteria (PGPR), which are among the diverse soil microflora, play a significant role in supporting plant development by having a variety of advantageous effects. This is frequently accomplished through the development of biofilms in the rhizosphere, which has benefits over planktonic bacterial life. However, prior studies have ignored the PGPR's ability to create biofilms. In contrast to the planktonic lifestyle of PGPR, this chapter focuses on novel ideas and insights regarding enhanced PGPR effects brought about by the biofilm formation by PGPR and its impact on promoting overall plant growth. Through quorum sensing in their biofilm state, advantageous PGPR is crucial to agricultural methods. Through a variety of plant growth processes, the in vitro production of biofilm PGPR can be used to boost crop yields. Through increased N2 fixation and uptake of micro- and macronutrients, they can be used as biofertilizers. Additionally, due to their capacity to produce plant growth regulators and function as biocontrol agents by producing antibiotics and other antimicrobial compounds, PGPR has been found to produce higher levels of plant

growth. The development of biofilmed PGPR with N2 fixing microbes would also be extremely advantageous to the microbial inoculant business. Biofilmed PGPR is an area that requires further investigation into its potential because it can be used to accomplish results in novel agricultural endeavours [4].

Although Bacillus subtilis and other Bacilli have long been used as biological control agents against bacterial plant diseases, it is unclear how exactly the bacteria provide that defense. In this research, we sought to identify *B. subtilis* strains from natural environments that have high biocontrol efficacy levels and to explore the mechanisms by which these strains give plant protection. Six strains that demonstrated above 50% biocontrol efficacy on tomato plants against the plant pathogen *Ralstonia solanacearum* under greenhouse conditions were found after screening a total of 60 isolates gathered from different places throughout China. These untamed strains demonstrated powerful antagonistic activities against different plant pathogens in plate assays and were capable of forming solid biofilms on tomato plant roots as well as in a defined medium. We demonstrate that the ability of those strains to protect plants was dependent on broadly conserved genes necessary for the formation of biofilms, including regulatory genes and genes that produce matrix. We offer proof that suggests matrix formation is essential for bacterial colonization on plant root surfaces. Finally, we have developed a model system for research on the interactions between *B. subtilis* and tomato plants in defense against a plant pathogen[5].

Due to its capacity to both encourage plant development and protect roots from biotic and abiotic stresses, *Stenotrophomonas rhizophila* has significant potential for applications in biotechnology and biological control. However, little is known about the mode of interactions in the root-environment system. Using transcriptomic and microscopic techniques, we investigated the processes underlying osmotic stress. The transcriptome of *S. rhizophila* DSM14405T significantly altered in reaction to salt or root extracts. For several functional gene families involved in general stress defense, energy production, and cell motility, we discovered a strikingly similar reaction. However, unique changes in the transcriptome were also observed: the negative regulation of flagella-coding genes together with the up-regulation of the genes responsible for biofilm formation and alginate biosynthesis were identified as a single mechanism of *S. rhizophila* DSM14405T against salt shock. However, glucosylglycerol (GG) production and excretion were discovered as remarkable methods for this Stenotrophomonas strain's stress defense. The switch from a planktonic to a sessile lifestyle in *S. rhizophila* treated with root exudates was detected as a down-regulation of flagellar-driven movement. These results are consistent with the observed positive modulation of host colonization genes and the diverse colonization patterns of oilseed rape roots seen in microscopic images. In addition to being a plant development regulator, spermidine has recently been discovered to be a stress-relieving agent. Overall, we were able to pinpoint the methods Stenotrophomonas uses to defend its roots from osmotic stress. Phytohormones and osmoprotectants have also been discovered to be important in stress protection, in addition to changes in lifestyle and energy metabolism[6].

Several major issues are brought on by bacterial biofilms in industrial fluid processing processes. Billions of dollars are lost annually due to mechanical obstructions, heat transmission process impediments, and the biodeterioration of metallic and polymeric system components. Contamination caused by biofilms can also lead to product spoilage and potential health risks for the general population. Fundamentally, the physicochemical characteristics connected to bacterial metabolism and biofilm formation can be used to characterize these biofouling activities. The distinctive structural characteristics of biofilms make the treatment of biofouling difficult because extracellular polymeric materials act as

diffusional barriers to antimicrobial agents, shielding labile cellular targets from both oxidizing and nonoxidizing chemicals. Weakly understood processes underlie the initial bacterial adhesion to engineered surfaces and the subsequent fouling of biofilm formation. However, the use of confocal laser microscopy, scanning or transmission electron microscopy, and Fourier transform infrared spectroscopy has significantly aided studies of bacterial biofilm architecture. The history of biofilm development is discussed in this paper, along with the impact of structure on biofouling processes in industrial fluid handling systems[7].

The opportunistic nosocomial pathogen Acinetobacter baumannii progressively becomes more prevalent in the clinical setting. It is challenging to eradicate these bacteria due to the high level of resistance mechanisms they have developed, one of which is the ability to create biofilms. A biofilm is made up of a dense community of bacteria that are held together by an extracellular matrix. (ECM). Bacterial exopolysaccharides (EPS), proteins, extracellular-DNA (e-DNA), and infrequently amyloidogenic proteins are among the polymers found in the extracellular matrix (ECM). The underlying bacterial community is protected by biofilm from the host immune system and chemotherapeutic agents. Therefore, current efforts are concentrated on finding a novel therapeutic that specifically targets infections linked with biofilms. Numerous illnesses can be treated naturally with plants. We have concentrated on natural herbal active substances in our search for an antibacterial drug substitute. In this research, we isolated active substances from different medicinal plants and tested their anti-biofilm efficacy against a strain of A. baumannii that was resistant to carbapenem. The results demonstrated that the polar extracts of clove (*Syzygium aromaticum*) and kiwi (*Actinidia deliciosa*) have potent anti-biofilm properties. The TLC profiling and phytochemical screening of these two plants were also used to identify the constituent secondary Agents the Biofilms. Sanguinarine, an alkaloid found in Actinidia deliciosa substance, is also a flavonoid. (hydroxyflavone). This extract's anti-biofilm impact on the *A. baumannii* ECM revealed that it lowers the levels of EPS, protein, and eDNA there. Because of their interactions with Congo Red, ECM proteins have also been shown to create amyloid-like structures. The findings were also supported by CFU counting following Actinidia deliciosa extract treatment. The polar extract of *A. deliciosa* can therefore be used to discover an effective alternative treatment to prevent the growth of biofilms caused by a carbapenem-resistant strain of Acinetobacter baumannii[8].

Environmental pollution from heavy metals has grown to be a serious problem for both the ecosystem and human health as a result of ongoing urbanization and industrialization processes. Dense ground-based communities of microbes called microbial biofilms are kept together by self-formed polymer matrixes, which are primarily comprised of polysaccharides, protein complexes, and extracellular DNAs. Microbes are used in phytoremediation, a long-term, cost-effective method for cleaning up and degrading a variety of toxic contaminants into less dangerous substances. Microbes in biofilm mode are advantageous for bioremediation due to their increased resistance to contaminants, reduced sensitivity to environmental stress, and capacity to break down a wide range of extremely harmful pollutants via different catabolic pathways. Complex molecules are reduced to simpler nutrients by the interplay of microbes and plants. Metal ions are also mobilized, and the bioaccumulation of contaminants is facilitated. In the biofilm state, microbes are contained in a self-produced matrix that serves as a barrier against stress and pollutants. Microbial biofilm consortia have been successfully used to eliminate all toxins, including heavy metals. The business uses microbe bioremediation to purify contaminated water and surfaces. Microbes that create biofilms are used for sewage treatment, biosorption, and plant protection. Biofilm formation may be aided by the use of adhesive surfaces, environmental variables, and

quorum-sensing molecules. Plant growth-promoting bacteria like Rhizobium, Bacillus, and Pseudomonas create biofilms on plant surfaces and in the soil thanks to advancements in microbe biofilm formation. This study covers the causes of microbial biofilm formation, the advantages of biofilms in phytoremediation, and the potential applications of biofilms in environmental cleanup techniques.

Despite having a wide variety of hosts and promoting plant growth, *Paenibacillus polymyxa* has not yet proven to be an effective biocontrol agent. Earlier research we conducted demonstrated that this bacteria shields Arabidopsis thaliana from pathogens and abiotic stress. Here, we investigated the colonization of plant roots by a *P. polymyxa* natural strain that had been marked with a gfp gene from a plasmid. Both electron scanning microscope and fluorescence microscopy revealed that the bacteria mostly colonized the root tip, where they created biofilms. In the intercellular gaps outside the vascular cylinder, bacteria accumulated. The absence of bacteria in aerial organs indicates that systemic spreading did not take place. Studies were conducted in both an earth system and a gnotobiotic system. Similar findings in both systems indicate that a more defined system can be used to study this bacterium's colonization. The negative effects of the bacteria that promote plant development are addressed, as well as issues with the green fluorescent protein (GFP) tagging of natural isolates[9].

CONCLUSION

The need for their protection from a variety of natural competitors, such as bacteria, fungi, insects, and other plants, arises from the intensive cultivation of plants in the monoculture field system to support the constantly expanding human population. The increased use of chemicals in the 20th century led to the development of many successful agricultural remedies.

However, finding a substance that would be effective only against a particular plant pathogen and be safe for the environment was very challenging. Scientists first tried to safeguard plants by using the natural competition between residing soil organisms in the late 1900s. Biocontrol was given to this occurrence. A very promising option to the prolonged use of pesticides—which are frequently expensive, build up in plants or soil, and have negative effects on people—is the biological control of plants by microorganisms. In addition to enhancing higher plants' capacity for adaptation, nonpathogenic soil bacteria can also be advantageous for their development. Here, we outline the situation concerning using *Bacillus subtilis* for biocontrol. This common soil dweller is generally acknowledged as a potential biocontrol agent. *B. subtilis*, which is naturally found in the vicinity of plant roots, is able to maintain steady contact with higher plants and encourage their development. In addition, *B. subtilis* and other Bacilli may be helpful as biocontrol agents because of their wide host range, capacity to form endospores, and ability to produce a variety of biologically active compounds with a wide range of activity.

REFERENCES:

[1] F. Villa, F. Cappitelli, P. Cortesi, and A. Kunova, “Fungal biofilms: Targets for the development of novel strategies in plant disease management,” *Front. Microbiol.*, 2017, doi: 10.3389/fmicb.2017.00654.

[2] C. Pandin, D. Le Coq, A. Canette, S. Aymerich, and R. Briandet, “Should the biofilm mode of life be taken into consideration for microbial biocontrol agents?,” *Microbial Biotechnology*. 2017. doi: 10.1111/1751-7915.12693.

[3] D. Debois, E. Jourdan, N. Smargiasso, P. Thonart, E. De Pauw, and M. Ongena, "Spatiotemporal monitoring of the antibiome secreted by bacillus biofilms on plant roots using MALDI mass spectrometry imaging," *Anal. Chem.*, 2014, doi: 10.1021/ac500290s.

[4] G. Seneviratne, M. L. M. A. W. Weerasekara, K. A. C. N. Seneviratne, J. S. Zavahir, M. L. Kecskés, and I. R. Kennedy, "Importance of Biofilm Formation in Plant Growth Promoting Rhizobacterial Action," 2010. doi: 10.1007/978-3-642-13612-2_4.

[5] Y. Chen *et al.*, "Biocontrol of tomato wilt disease by Bacillus subtilis isolates from natural environments depends on conserved genes mediating biofilm formation," *Environ. Microbiol.*, 2013, doi: 10.1111/j.1462-2920.2012.02860.x.

[6] P. Alavi, M. R. Starcher, C. Zachow, H. Müller, and G. Berg, "Root-microbe systems: The effect and mode of interaction of stress protecting agent (SPA) Stenotrophomonas rhizophila DSM14405T," *Front. Plant Sci.*, 2013, doi: 10.3389/fpls.2013.00141.

[7] M. W. Mittelman, "Structure and Functional Characteristics of Bacterial Biofilms in Fluid Processing Operations," *J. Dairy Sci.*, 1998, doi: 10.3168/jds.S0022-0302(98)75833-3.

[8] V. Tiwari, D. Tiwari, V. Patel, and M. Tiwari, "Effect of secondary metabolite of Actinidia deliciosa on the biofilm and extra-cellular matrix components of Acinetobacter baumannii," *Microb. Pathog.*, 2017, doi: 10.1016/j.micpath.2017.07.013.

[9] S. Timmusk, N. Grantcharova, and E. G. H. Wagner, "Paenibacillus polymyxa invades plant roots and forms biofilms," *Appl. Environ. Microbiol.*, 2005, doi: 10.1128/AEM.71.11.7292-7300.2005.

CHAPTER 10

COMPOUNDS USED AS ANTIMICROBIAL AGENTSINBIOFILMS

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ABSTRACT:

Due to the emergence of multidrug-resistant strains, biofilm formation in clinical settings is becoming an increasingly significant problem. This increased mortality places a significant financial burden on healthcare systems. To effectively manage infections brought on by biofilm-forming microbes, novel strategies are wanted in addition to conventional antibiotics. This is because bacterial biofilms are quite resistant to routine antimicrobial-based therapies. Biofilm inhibitors and modified biomaterials for the development of medical devices to prevent biofilm formation are currently the methods being proposed to control the formation of biofilms in clinical practice settings. In this chapter, we've concentrated on the most recent advancements in anti-biofilm tactics by disrupting the quorum-sensing system, which is essential for biofilm formation, and we've compiled a list of the different antibacterial compound classes that can be used to prevent the growth of biofilms.

KEYWORDS:

Antimicrobial Compounds, Biofilm Formation, Essential Oil, Medical Device, Planktonic Cells.

INTRODUCTION

A population of bacteria known as a biofilm is affixed to a surface or substrate. In a biofilm, bacteria are embedded in an extracellular polymeric matrix that the bacteria have created. On submerged surfaces, including natural aquatic systems, water pipelines, living tissues, tooth surfaces, indwelling medical devices, and implants, bacteria form biofilms. A serious medical issue is the development of biofilms on indwelling medical implants and devices like catheters, artificial heart valves, pacemakers, prosthetic joints, and contact lenses. On indwelling medical devices, both Gram-negative and Gram-positive bacteria can create biofilms. The most frequent bacteria that create biofilms are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans*, and *Enterococcus faecalis. S. aureus* and *S. epidermidis* are the two types of these biofilm-forming bacteria that are most frequently found on cardiovascular devices.

According to estimates, S. aureus and *S. epidermidis* were to blame for 40%–50% of infections in prosthetic heart valves and 50%–70% of catheter biofilm infections. The 150 million intravascular devices inserted in the US each year experience between 250,000 and 500,000 primary bloodstream infections. For each infection, the expense of healthcare could rise from $4000 to $56,000. Staphylococci accounted for about 87% of bloodstream illnesses. Together, *S. aureus* and *S. epidermidis* in biofilm place a tremendous load on the healthcare system [1]. Microorganisms that are floating freely can attach to a surface to create biofilms. Although biofilms have some advantageous uses, they are generally regarded as undesirable, and strategies for preventing biofilm formation have been developed. The means of prevention have thus primarily focused on two areas: killing the microbes that create the film or preventing the adhesion of the microbes to a surface.

Biofilms secrete an extracellular polymeric substance that serves as a structural matrix and promotes adhesion for the microorganisms. Because biofilms serve as a form of bacterial defense, they are frequently more resistant to conventional antimicrobial treatments, posing a serious threat to human health. For instance, more than one million cases of catheter-associated urinary tract infections (CAUTI) are recorded each year, and bacterial biofilms play a significant role in many of these cases.

The prevention of biofilms is the subject of extensive research. The primary method for preventing biofilm on indwelling medical devices is chemical modification. Chemical methods of preventing biofilms are frequently employed, including antibiotics, biocides, and ion coverings. By obstructing the attachment and growth of immature biofilms, they prevent the development of biofilms. These coatings typically only work for a short time (about a week), after which the antimicrobial agent starts to leach out and decrease the coating's efficacy. Since the Phoenicians used silver bottles to hold their water, wine, and vinegar to prevent them from going bad, silver and silver ions have been used for medical purposes. For antimicrobial reasons, silver coatings are once again gaining popularity.

Agent	Mechanism	Effect
Anti-virulence compounds	Inhibition of gene expression of virulence factors	Inhibition of biofilm formation by *S. aureus*
Anti-biofilm compounds	Unknown	Inhibition of biofilm formation by *S. epidermidis*
ABC-1	Inhibition of c-di-GMP-inducible transcription	Inhibition of biofilm formation by multiple Gram-negative and Gram-positive bacterial pathogens
Aryl rhodanines	Unknown	Inhibition of biofilm formation by *S. aureus* and *S. epidermidis*
Cis-2-decenoic acid	Unknown	Dispersion of biofilms by *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. pyogenes*, *B. subtilis*, *S. aureus*, and *C. albicans*
D-amino acids	Unknown	Inhibition of biofilm formation by *S. aureus* and *P. aeruginosa*
N-acetylcysteine	Interference with exopolysaccharide formation in biofilms	Inhibition of biofilm formation by *S. epidermidis*
Chelators	Interference with metal ion's function in biofilm formation	Inhibition of biofilm formation by *S. aureus*

Figure 1: Antimicrobial agents: Figure showing the different antimicrobial agents used against the microorganism (Semantic scholar).

The oligodynamic effect, which is a process in which metal ions prevent bacteria from growing and functioning normally, is what gives silver its antibacterial properties. Silver is successful at preventing infection in several in vitro studies, both in coating form and as nanoppapers dispersed in a polymer matrix. The use of silver in vivo, however, continues to raise questions. Some people worry that silver may have a toxic effect on human tissue due to the method by which it affects bacterial cell function. Silver compounds have only been used sparingly in vivo due to this. Despite this, silver finishes are frequently applied to tools like catheters. (Figure .1) depicts some key chemicals that prevent biofilms. Biofilms can become weakened and dispersed if their extracellular polymeric framework is disrupted or degraded (Figure .1).

Coating agent	Coating method	Mechanism
Antibiotics	Non-covalent, covalent bonding	Bactericidal/Bacteriostatic
Silver	Plasma deposition, sol-gel coating, wet-chemical coating	Bactericidal
Furanones	Physical adsorption, covalent bonding	Bactericidal/Bacteriostatic
QAS	Covalent bonding	Inhibition of bacterial adhesion and viability
Silica nanoparticles with QAS	Covalent bonding	Bactericidal/Bacteriostatic
TMS	Plasma coating deposition with covalent bonding	Anti-adhesion
PLL-g-PEG	Physical adsorption & covalent coupling	Anti-adhesion
pCBMA	Zwitterionic surfaces grafted via radical polymerization	Anti-adhesion
Silica colloids/Silane xerogel	Synthesis of superhydrophobic coating	Anti-adhesion
Submicron surface textures	Physical surface roughness modification	Anti-adhesion
Selenocyanatodiacetic acid	Covalent bonding	Anti-adhesion
Polymer brush coatings	Surface grafting	Anti-adhesion

Figure 2: Surface modification agents: Diagram showing the different surface modifying agents for inhibits the growth of the biofilms formation (Semantic scholar).

Studies have been conducted to break down proteins, eDNA, and polysaccharides that are part of the matrix. *Actinobacillus actinomycetemcomitans*, a Gram-negative oral bacterium, makes dispersin B, which can break up bacterial biofilms. Dispersin B was discovered to be able to damage the extracellular matrix of the *S. epidermidis* biofilm and disperse it, according to Kaplan et al. Bacteria produce extracellular genomic DNA (eDNA), which is a crucial part of the biofilm's extracellular matrix. DNase I was found to have the ability to break up S. aureus biofilms as a consequence. S. aureus biofilms were successfully dislodged by trypsin and proteinase K.

These methods continue to have many shortcomings. Such methods' in vivo effectiveness isn't well proven, and treating the host with proteins might result in an inflammatory or allergic reaction, which might have an impact on their therapeutic potential [1]. For industries like medicine, dentistry, food processing, and water purification that directly impact human health and life, preventing bacterial biofilm formation is crucial. This study demonstrates an efficient and cost-effective method for reducing attachment and biofilm formation by several pathogenic bacteria frequently linked to medical infections and foodborne illnesses. In conclusion, treating biofilm-related infections is preferable to preventing biofilm formation, which is possible with the bioengineering methods (Figure. 2). The most successful and promising method for reducing the morbidity and mortality caused by biofilm infections, despite the drawbacks of many methods, is to enhance the anti-biofilm properties of biomaterials.

Numerous plant extracts and their compounds have been extensively researched to remove the "Propionibacterium acne" biofilm. Five plant extracts, including *Rhodiola crenulata*, Dolichos lablab, *Malus pumila*, *Epimedium brevicornum*, and *Polygonum cuspidatum*, were found to have significant antibiofilm activity. Additionally, according to these researchers, even when used at the lowest MIC, extracts of *P. cuspidatum* and *E. brevicornum* as well as their active compounds, resveratrol and icartin, may have antibiofilm properties. *Melia dubia* bark preparations at a concentration of 30 mg/mL were assessed. Additionally, these extracts show promise for suppressing E. coli hemolysis, swarming motility, hydrophobicity, and biofilm production. EPS production and biofilm production are inhibited by *Capparis*

spinosa (caper bush) extract in *Serratia marcescens*, *Pseudomonas aeruginosa*, Proteus mirabilis, and Escherichia coli at 2 mg/mL concentration, according to similar findings from other peers. Additionally, three microorganisms' well-known biofilm formation was scattered. Fruit extract from the therapeutically significant plant "Lagerstroemia speciosa," which is typically found in Southeast Asia, can prevent "*P. aeruginosa*" PAO1 from forming biofilms when used at a concentration of 10 mg/mL. Essential oils are naturally occurring volatile substances obtained from plants. (EOs). These natural products are beneficial to the food business and effective due to their antibacterial and preservative qualities. Since early times, these essential oils have been used frequently to combat a variety of microorganisms. These oils have an antimicrobial effect on microorganisms' cell walls, which causes the microbes to die. Additionally, it is claimed that these oils are highly effective at killing a variety of microorganisms without causing antimicrobial tolerance [2].

LITERATURE REVIEW

Due to the presence and ongoing evolution of resistant microorganisms and phenotypes, the emergence of new diseases, and the toxicity of some existing antimicrobials, it is necessary to identify new sources of antimicrobial products. The development of bacterial resistance, including multidrug resistance (MDR), is inevitable because it is a specific facet of microbial evolution as a whole. It is necessary to find and create new goods because bacterial resistance to traditional antimicrobials is on the rise. In this situation, phytochemicals have already shown that they have the ability to work both independently as antibacterials and in concert with other, less potent antibacterials to increase their efficacy. Furthermore, new research has shown that phytochemicals can be used in the management of biofilms and in situations where bacterial resistance mechanisms, such as MDR, render conventional treatments ineffective. This review's objective is to outline recent developments in phytochemical antibiotic activities and their mechanisms of action while also highlighting recent advancements in their management of MDR bacteria and biofilms[3].

The most important bacterium in the transformation of commensal, non-pathogenic oral microbiota into biofilms that aid in the development of tooth caries is thought to be *streptococcus mutans*. The objective of the current research was to assess the antimicrobial activity of cinnamaldehyde, a naturally occurring plant product, against *S. mutans* biofilms. To evaluate its antimicrobial action against planktonic *S. mutans*, minimum inhibitory concentrations (MIC), minimal bactericidal concentrations (MBC), and growth curves were calculated. The crystal violet and MTT tests were used to measure the biomass and metabolism of the biofilms at various cinnamaldehyde concentrations and incubation times. With the aid of a confocal laser scanning microscope, the biofilms were seen. (CLSM). After cinnamaldehyde therapy, bacterial cell surface hydrophobicity, aggregation, acid production, and acid tolerance were assessed.

Real-time PCR was used to examine the gene expression of virulence-related components (gtfB, gtfC, gtfD, gbpB, comDE, vicR, ciaH, ldh, and relA). For planktonic *S. mutans*, the MIC and MBC of cinnamaldehyde were 1000 and 2000 g/mL, respectively. Cinnamaldehyde can reduce biofilm biomass and metabolism at sub-MIC concentrations, according to the findings. According to CLSM images, the surface regions covered in biofilm shrank as cinnamaldehyde concentrations rose. Cinnamaldehyde improved acid tolerance and acid generation while decreasing *S. mutans* aggregation and increasing cell surface hydrophobicity. In the presence of cinnamaldehyde, gene expression in the biofilms was down-regulated. Our results thus showed that cinnamaldehyde at the sub-MIC level inhibited microbial activity on *S. mutans* biofilm by modulating gene expression for virulence, hydrophobicity, aggregation, acid production, and acid tolerance[4].

Abstract Antibiotic resistance is a problem because some bacterial infections are becoming increasingly difficult for medicines to treat. There is a critical need for new antibacterial substances. The best place to find novel antimicrobials is thought to be in plants. This research aimed to evaluate the antimicrobial activity of four phytochemicals against Escherichia coli and Staphylococcus aureus, either as planktonic cells or as biofilms: 7-hydroxycoumarin (7-HC), indole-3-carbinol (I3C), salicylic acid (SA), and saponin (SP). These bacteria are frequently discovered in infections obtained in hospitals. Investigations were done on a few aspects of the phytochemicals' mechanism of action, such as surface charge, hydrophobicity, motility, and quorum-sensing inhibition (QSI). To determine whether there was a synergistic impact, three antibiotics were added to the phytochemicals.

The most potent phytochemicals against E. coli and S. aureus were 7-HC and I3C. Both polyphenols had an impact on quorum-sensing (QS) and motility, suggesting that they may be crucial in inhibiting cell-cell communication as well as the development and management of biofilms. However, none of the chosen polyphenols were able to completely remove the biofilm. Tetracycline (TET), erythromycin (ERY), ciprofloxacin (CIP), and I3C in dual combinations had synergistic impacts on S. aureus resistant strains. The overall picture shows that phytochemicals have the ability to limit the development of S. aureus and E. coli in both planktonic and biofilm states. Additionally, the phytochemicals showed the potential to work in concert with antibiotics, aiding in the repurposing of antimicrobials that were previously thought to be useless due to resistance issues[5].

By adding gold nanoppapers stabilized by an ionic silsesquioxane that includes the 1, 4-diazoniabicyclo [2.2.2] octane chloride group, active biofilms of quinoa (*Chenopodium quinoa*, W.) starch were created. Escherichia coli and *Staphylococcus aureus* were tested against the biofilms to determine their antibacterial activity. When compared to the typical biofilm, the presence of gold nanoppapers improves the mechanical, optical, and morphological properties while keeping the thermal and barrier properties. With inhibition rates of 99% against E. coli and 98% against S. aureus, the active biofilms demonstrated potent antibacterial activity against food-borne pathogens. The use of these quinoa starch biofilms containing gold nanoppapers as active food packing for preserving food safety and extending the shelf life of packaged foods is very promising[6].

Identify the antimicrobial properties of contact lens cleaning solutions, as well as whether or not clinical and reference strains of *Pseudomonas aeruginosa*, Serratia marcescens, and *Staphylococcus aureus* creates biofilms on silicone hydrogel contact lenses. *Pseudomonas aeruginosa*, Serratia marcescens, and Staphylococcus aureus clinical and American Type Culture Collection (ATCC) reference isolates were incubated with lotrafilcon A lenses in a biofilm-forming environment. Scanning electron microscopy (SEM) and confocal microscopy were used to assess the gross morphology and design of biofilms, and colony forming units (CFUs) were used to quantify them. The susceptibilities of five popular multipurpose contact lens cleaning solutions and one hydrogen peroxide cleaning solution to the planktonic and biofilm growth stages of the bacteria were evaluated.

Reference and clinical isolates of *P. aeruginosa, S. marcescens*, and *S. aureus* developed biofilms on lotrafilcon. A silicone hydrogel contact lens has a visible extracellular matrix and dense networks of cells arranged in numerous layers. Commonly used biguanide-preserved multipurpose care products could not remove the biofilms. While *S. marcescens* biofilm was resistant to a polyquaternium-preserved care solution but vulnerable to hydrogen peroxide disinfection, *P. aeruginosa* and *S. aureus* biofilm were both susceptible to hydrogen peroxide and a polyquaternium preserved care solution. The planktonic species, however, were always vulnerable. Biofilms are created by P. aeruginosa, S. marcescens, and S. aureus on

lotrafilcon. Contrary to planktonic cells, contact lenses are immune to the antimicrobial effects of many soft contact lens maintenance products[7].

The antimicrobial properties of natural isothiocyanates (ITCs) found in plants such as nasturtium (Tropaeolum majus) and horseradish (Armoracia rusticana), and the need for new chemotherapeutic options for the treatment of infections caused by multidrug-resistant and biofilm-forming Gram-negative bacteria such as *Pseudomonas aeruginosa* (Pa), led us to evaluate the effects of three major ITCs, allylisothiocyanate (AITC), benzylisothiocyanate (BITC), and phenylethyl-isothiocyanate (PEITC), and a mixture (ITCM) adapted to the ITC composition after release of active components out of natural sources.

27 Pa samples with increased biofilm formation were chosen for testing out of 105 Pa isolates. The effects of ITCs on Pa were assessed concerning (1) the growth of planktonic bacteria, (2) the development of biofilms, (3) the metabolic activity of mature biofilms, and (4) the interaction between ITCs and antibiotics. (1) Anti-Pa action was present in each ITC. The following were the average minimal inhibitory concentrations (MICs): AITC 103 6.9; BITC 2145 249; PEITC 29 423 1652; and ITCM 140 5. (2) Biofilm formation was substantially reduced when bacteria were treated with PEITC and ITCM at concentrations below the MIC. In particular, ITCM decreased bacterial growth and biofilm density. (3) Mature biofilms' metabolic activity was markedly suppressed by ITCs. (4) Meropenem and ITCs together enhanced the antibacterial effectiveness against Pa biofilms. ITCs are a potential class of organic anti-infectives that are effective against Pa biofilms [8].

The purpose of the current research was to assess the ability of essential oils to eliminate *Staphylococcus aureus*, a foodborne pathogen, from food-processing facilities. The minimal inhibitory concentration was used to gauge the efficacy of 19 essential oils against planktonic S. aureus cells. The susceptibility of planktonic cells to essential oils varied greatly, with thyme oil being the most potent, followed by lemongrass oil and vetiver oil. The eight essential oils that were most successful at killing planktonic cells were then evaluated on 48-hour-old biofilms that had developed on stainless steel. None of the essential oils could fully eliminate biofilms, but they all substantially (p 0.01) decreased the number of viable biofilm cells. Although high concentrations were required to produce logarithmic reductions over 4 log CFU/cm2 after 30 min of exposure, thyme and patchouli oils were the most efficient. As an alternative, the use of sub-lethal amounts of thyme oil allowed for the slowing of biofilm development and the improvement of thyme oil and benzalkonium chloride's effectiveness against biofilms. There was evidence of some cellular response to thyme oil, though. To avoid the rise of strains that are resistant to antibiotics, essential oil-based treatments should be based on the alternation and combination of various essential oils or with other biocides [9].

Staphylococcus aureus and *Pseudomonas aeruginosa* biofilms were produced in an in vitro flatbed perfusion biofilm model. A static diffusion technique was used to expose mature biofilms to wound dressings containing either silver or iodine (Aquacel Ag and Iodozyme) for up to 24 hours. The key elements that determine antimicrobial activity in the wound were taken into consideration when developing this technique. Over the course of the test session, the numbers of viable bacteria residing in the biofilms were counted at predetermined intervals. Although the iodine dressing was more effective under the tested experimental conditions, both test dressings had an antimicrobial impact on the target species biofilms. The efficacy of wound dressings containing broad-spectrum antimicrobial agents, such as silver and iodine, against particular types of bacterial biofilms varies widely and possibly significantly (as measured in vitro)[10].

CONCLUSION

The significance of biofilms in hospitals, particularly concerning their function in infections, is frequently undervalued because of how common they are in nature. Future research should focus on understanding the biological factors governing colonization to create creative methods for reducing biofilm biomass in a therapeutic setting. The comprehensive study is also needed to understand the potential of different anti-microbial agents both natural and synthetic, for use against the microbes. These methods can undoubtedly serve as future therapeutic agents for the treatment of biofilm-based bacterial infections in clinical environments because they do not promote antibiotic resistance.

REFERENCES:

[1] M. Chen, Q. Yu, and H. Sun, "Novel strategies for the prevention and treatment of biofilm related infections," *International Journal of Molecular Sciences*. 2013. doi: 10.3390/ijms140918488.

[2] A. Shahid *et al.*, "Innovative Strategies for the Control of Biofilm Formation in Clinical Settings," in *Bacterial Biofilms*, 2020. doi: 10.5772/intechopen.89310.

[3] M. Simões, R. N. Bennett, and E. A. S. Rosa, "Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms," *Natural Product Reports*. 2009. doi: 10.1039/b821648g.

[4] Z. He, Z. Huang, W. Jiang, and W. Zhou, "Antimicrobial Activity of Cinnamaldehyde on Streptococcus mutans Biofilms," *Front. Microbiol.*, 2019, doi: 10.3389/fmicb.2019.02241.

[5] J. Monte, A. C. Abreu, A. Borges, L. C. Simões, and M. Simões, "Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms," *Pathogens*, 2014, doi: 10.3390/pathogens3020473.

[6] C. H. Pagno *et al.*, "Development of active biofilms of quinoa (Chenopodium quinoa W.) starch containing gold nanoppapers and evaluation of antimicrobial activity," *Food Chem.*, 2015, doi: 10.1016/j.foodchem.2014.10.068.

[7] L. B. Szczotka-Flynn *et al.*, "Increased resistance of contact lens-related bacterial biofilms to antimicrobial activity of soft contact lens care solutions," *Cornea*, 2009, doi: 10.1097/ICO.0b013e3181a81835.

[8] S. J. Kaiser, N. T. Mutters, B. Blessing, and F. Günther, "Natural isothiocyanates express antimicrobial activity against developing and mature biofilms of Pseudomonas aeruginosa," *Fitoterapia*, 2017, doi: 10.1016/j.fitote.2017.04.006.

[9] D. Vázquez-Sánchez, M. L. Cabo, and J. J. Rodríguez-Herrera, "Antimicrobial activity of essential oils against Staphylococcus aureus biofilms," *Food Sci. Technol. Int.*, 2015, doi: 10.1177/1082013214553996.

[10] R. M. Thorn, A. J. Austin, J. Greenman, J. P. Wilkins, and P. J. Davis, "In vitro comparison of antimicrobial activity of iodine and silver dressings against biofilms.," *J. Wound Care*, 2009, doi: 10.12968/jowc.2009.18.8.43635.

CHAPTER 11

DIFFERENT STAGES INVOLVED IN THE DEVELOPMENT OF THE BIOFILM IN THE NATURAL SYSTEM

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ABSTRACT:

The biofilm may be defined as a microbe-derived sessile community described by living things that are connected to a substratum, interface, or each other are rooted in a matrix of extracellular polymeric material, and exhibit an influenced phenotype concerning growth, gene expression, and protein production. The stages of the biofilm infection life cycle are typically attachment the interaction of bacteria with the implant, accumulation the interaction of bacteria, maturation the creation of a viable 3D structure, and dispersion/detachment. Release from the biofilm. Depending on the creature involved, the life cycle of a biofilm varies. There are traits in the biofilm development life cycle. Attachment, proliferation/accumulation/maturation, and dispersion are some of these. Biofilm can be discovered as floating aggregates or adherent to a surface. For the creation of therapeutic strategies targeted at preventing, interrupting, and eliminating biofilm-associated infections, it is crucial to comprehend the progression of biofilm life cycles and the processes that pathogens use to control this progression.

KEYWORDS:

Bacterial Biofilms, Life Cycle, Planktonic Cells, Species Biofilms, Wild Type.

INTRODUCTION

Microorganisms that can develop on a variety of surfaces come together to form biofilms. The bacteria, fungi, and protists that create biofilms are microorganisms. Biofilms are intricate multicellular formations made by bacteria [1]. There are generally accepted to be four main phases in the formation of biofilms: (1) bacterial attachment to a surface, (2) microcolony formation, (3) biofilm maturation, and (4) detachment (also known as dispersal) of bacteria that may then colonize new areas (Figure .1). Sessile bacteria, also known as biofilm bacteria, have phenotypes that are different from planktonic bacteria and live in a stationary or dormant growth phase. Bacteria in biofilms exhibit extraordinary resilience to environmental stresses, particularly antibiotics. As a result, biofilms pose a serious threat to public health because they are responsible for 60 to 80 percent of human microbial illnesses. To stop the development of biofilms, it is crucial to identify the biochemical processes and biological elements that are essential. Even though we have a general grasp of the structure and growth of bacterial biofilms, we still don't fully understand the mechanisms that trigger the change from planktonic to sessile cells. A phenotypic change is believed to result from this transition, which is thought to be a complicated and tightly controlled process [1].

The microbial cells that form a biofilm are physiologically different from the planktonic cells of the same organism, which are individual cells that can float or move in liquid but are otherwise the same organism. Numerous variables can cause microbes to create a biofilm, such as cellular recognition of particular or general attachment sites on a surface, dietary cues, or exposure of planktonic cells to sub-inhibitory antibiotic concentrations. Large suites

of genes are differentially regulated when a cell transitions to the biofilm phase of development, causing a phenotypic change in behavior.

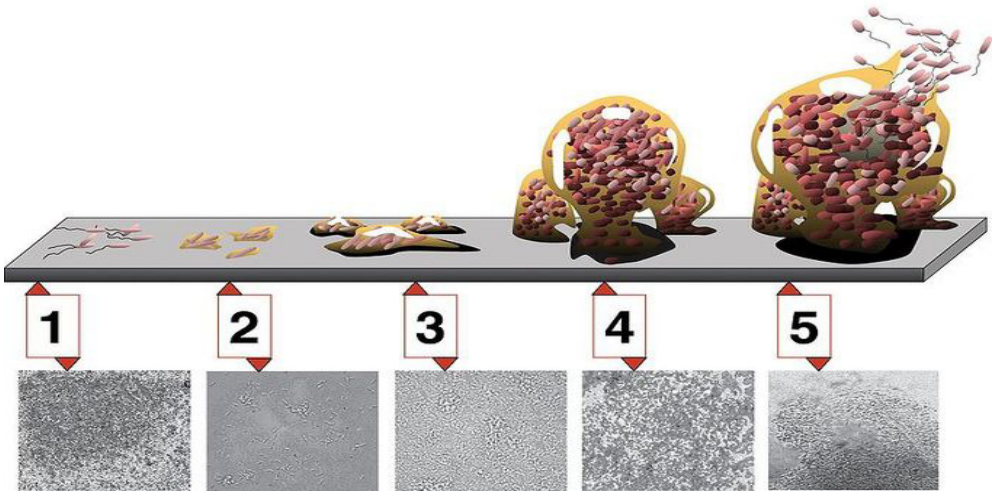

Figure 1.Stages of biofilms developments: Diagrams showing the different stages of the biofilms developments (Wikipedia).

Free-floating microbes cling to a surface to start the formation of a biofilm. Through van der Waals forces, these first colonizers initially establish a weak, reversible adhesion to the surface. If the colonists are not instantly severed from the surface, they can use cell adhesion structures like pili to more firmly anchor themselves. Some species can anchor themselves to the matrix or directly to previous colonists even though they are unable to attach to a surface on their own. The cells are able to interact during this colonization by using quorum-sensing products like AHL.

The biofilm expands once colonization has started through a mix of cell division and recruitment. Development is the last phase of biofilm creation; during this phase, the biofilm is established and only undergoes minor shape and size changes. The emergence of a biofilm may facilitate the formation of an antibiotic-resistant aggregate cell colony (or colonies).

One crucial phase of the biofilm life cycle is the dispersion of cells from the biofilm population. Biofilms can expand and colonize new surfaces through dispersal (Figure.2). Biofilm dissemination may be aided by enzymes that break down the extracellular matrix of biofilms, like deoxyribonuclease and dispersion B. As anti-biofilm compounds, enzymes that break down the biofilm matrix might be helpful. There is proof that the fatty acid messenger cis-2-decenoic acid can cause biofilm populations to disperse and stop growing. This substance, which is secreted by *Pseudomonas aeruginosa*, causes cyclo heteromorphic cells in several bacterial species and the yeast Candida albicans. Additionally, it has been demonstrated that nitric oxide causes various bacterial species' biofilms to spread. at levels below the hazardous threshold. Nitric oxide may be used to help patients with chronic infections brought on by biofilms.

Most people believed that when cells are released from biofilms, they instantly enter the planktonic growth phase. However, research has revealed that the physiology of *Pseudomonas aeruginosa* biofilm-distributed cells differs significantly from that of planktonic and biofilm cells. Therefore, the dispersal process is a special phase in the shift of bacteria from a biofilm to a planktonic lifestyle. When compared to planktonic cells, dispersed cells are found to be significantly more virulent against macrophages and *Caenorhabditis elegans*, but significantly more susceptible to iron stress.

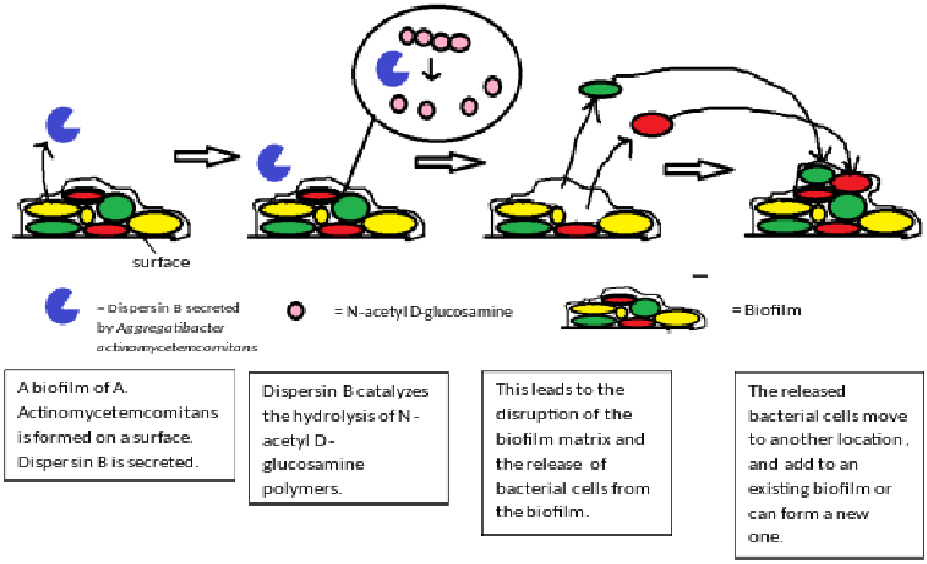

Figure 2: Biofilms dispersal: Diagram showing the dispersal mechanism of the bacteria biofilms (Wikipedia).

Although they can develop as floating mats on liquid surfaces and the surface of leaves, especially in high-humidity regions, biofilms are typically found on solid substrates submerged in or exposed to some aqueous solution. A biofilm will rapidly reach macroscopic size if given enough resources for growth. Microorganisms of many various types, including bacteria, archaea, protozoa, fungi, and algae, can be found in biofilms, each of which performs a specific metabolic task. However, under specific circumstances, some organisms will create monospecies films. Chronic opportunistic infections, which have become more prevalent in immunocompromised patients and the aging population, severely hinder medical advancements in industrialized societies.

The medical community continues to face significant challenges from chronic infections, which also have significant fiscal implications because they are frequently resistant to conventional antibiotic therapy. The capacity of the bacteria to grow within biofilms that shield them from harmful environmental factors appears to be one of the main causes of persistence. In addition to being a significant opportunistic pathogen and the origin of emerging nosocomial infections, *Pseudomonas aeruginosa* serves as a model organism for the investigation of a variety of bacterial processes involved in bacterial persistence. In this context, the elucidation of the molecular mechanisms responsible for the switch from planktonic growth to a biofilm phenotype and the role of inter-bacterial communication in persistent disease should provide new insights into *P. aeruginosa* pathogenicity, contributes to better clinical management of chronically infected patients and should lead to the identification of new drug targets for the development of alternative anti-infective treatment strategies Antibiotic-resistant bacteria are those that are linked with biofilms.

The extracellular polymeric matrix and complex biofilm structure may make it difficult for medicines to reach the bacteria. Due to the altered microenvironment, which includes nutrient depletion and waste accumulation, bacteria in biofilm may also assume a slow-growing or starved condition. Bacteria may become more resistant to antibiotics, which target more active cell processes, as a result of their altered physiological condition. The goals of this chapter are to emphasize [2] and provide an overview of biofilm formation and surface adherence.

LITERATURE REVIEW

Free-swimming cell attachment to a surface occurs throughout the formation of a biofilm, first briefly and then permanently as a single layer. A three-dimensional structure made up of massive bacterial pillars and water channels, the biofilm ultimately develops from this monolayer of immobilized cells into larger cell clusters. Previous research has demonstrated that pili, flagella, and exopolysaccharide must all be present for the *Vibrio cholerae* biofilm to form effectively. The prerequisites for monolayer creation by wild-type *V. cholerae* are, however, poorly understood. In this study, we isolated the wild-type *V. cholerae* monolayer and showed that distinct environmental cues, bacterial features, and transcriptional patterns are required to initiate and maintain the monolayer state. Monolayer cells are designed specifically to keep their adhesion to a surface. Flagellar gene transcription is suppressed when the surface itself triggers mannose-sensitive haemagglutinin type IV pilus (MSHA)-mediated attachment. Cells in a biofilm, on the other hand, are trained to sustain intercellular contacts. When exopolysaccharide synthesis is stimulated by environmental monosaccharides, the process advances to this step. In our model, cells create a stable monolayer on a surface and biofilms develop in natural settings. The monolayer transforms into a biofilm as biotic surfaces deteriorate with the following release of carbohydrates [3].

In the past, scientists have examined bacterial signaling as if it consisted of discrete, linear paths. However, more recent research has shown that a variety of signaling pathways communicate with one another and that this network of interconnected pathways is complex. For the right subset of genes to be expressed in the right quantity at the right moment, this network integrates a variety of extracellular and intracellular signals. Major objectives of systems biology include the thorough delineation of this intricate signal transduction network and the use of the network to forecast the full spectrum of cellular behaviors. We are still in the early stages of this process, which has so far been driven by the creation of enabling technologies and the assembling of gene lists, despite making significant progress. The next crucial step must be to arrange the copious data gathered over five decades of pregenomics research and the enormous amount of postgenomics data produced over the past decade, even though development and compilation will continue to be crucial.

This minireview is an effort to carry out a portion of the following step [4] in which we describe a portion of the overall network of Escherichia coli. The use of *Pseudomonas aeruginosa* as a model organism for biofilm development and pathogenesis has regained popularity over the past ten years. Since the biofilm matrix serves as a vital interface between the bacterium and the host or its environment, a great deal of effort has been put into comprehending the matrix's makeup more thoroughly. The functions of alginate, Psl, and Pel polysaccharides in the biofilm matrix are the main topics of this paper [5]. In reaction to the right environmental cues, planktonic cells interact with a surface to create complex bacterial communities known as biofilms. We describe the isolation and characterization of mutants of *Pseudomonas aeruginosa* PA14 that are ineffective at establishing biofilms on a polyvinylchloride (PVC) plastic surface.

Surface attachment defective is the label given to these mutations (sad). The analysis of two groups of sad mutants included (i) mutants with defects in flagellar-mediated motility and (ii) mutants with defects in the polar-localized type IV pili's biogenesis. Using phase-contrast imaging, we monitored the growth of the biofilm produced by the wild type over 8 hours. The abiotic surface of the wild-type strain first developed a monolayer of cells, after which microcolonies that were distributed throughout the monolayer of cells emerged. We show proof that microcolonies form when cells in the monolayer aggregate using time-lapse microscopy. On the PVC plastic, strains with mutations in genes essential for the synthesis of

type IV pili created a monolayer of cells, just as was seen with the wild type. The type IV pili mutants did not, however, create microcolonies throughout the experiments, in contrast to the wild-type strain, indicating that these structures play a significant role in microcolony formation.

Even after 8 hours of incubation, only a small number of cells from a non-motile strain (carrying a mutation in flgK) adhered to PVC, indicating a function for flagella and/or motility in the early cell-to-surface interactions. Thus, we can start dissecting the developmental process leading to biofilm formation thanks to the phenotype of these mutants [6]. *Candida albicans* produce the quorum-sensing compounds tyrosol and farnesol, which respectively speed up and prevent the morphological change from yeasts to hyphae. In this research, we looked into the tyrosol secretion by *Candida albicans* and speculated on its potential function in the formation of biofilms. Four different strains of Candida albicans, including three mutants with clear deficiencies in the Efg 1 and Cph 1 morphogenetic signaling pathways, produced extracellular tyrosol during development at 37°C in both planktonic (suspended) cells and biofilms.

For both cell groups, there was a connection between tyrosol synthesis and biomass. However, when tyrosol production was correlated to cell dry weight, biofilm cells produced at least 50% more tyrosol than planktonic cells. After 48 hours, an exogenous farnesol addition to a wild-type strain reduced biofilm development by up to 33%. Tyrosol from exogenous sources didn't seem to have any impact, but scanning electron microscopy demonstrated that it stimulated the growth of hyphae in the early (1 to 6 h) phases of biofilm development. Tyrosol and farnesol were added simultaneously in experiments at varying concentrations, and the 48-hour biofilms that resulted contained almost exclusively yeast cells, indicating that farnesol's activity was dominant.

When biofilm supernatants were tested for their abilities to inhibit or enhance germ tube formation by planktonic cells, the results indicated that tyrosol activity exceeds that of farnesol after 14 h, but not after 24 h, and that farnesol activity increases significantly during the later stages (48 to 72 h) of biofilm development. Overall, our findings are consistent with the idea that tyrosol functions as a quorum-sensing molecule for both planktonic cells and biofilms, with its influence being greatest in the early and intermediate phases of biofilm formation [7]. Our current models of bacterial biofilm formation were built using experimental methods that mainly utilized genetic and microscopic tools. This study has made it possible for researchers to categorize the formation of biofilms into distinct stages. The original attachment of microbes to a surface or one another, the development of microcolonies, the maturation of the biofilm, and its dispersal are all thought to be part of the biofilm developmental cycle. Bacterial physiology and phenotypic responses that are specific to the various biofilm stages indicate the existence of distinct biofilm biology distinct from that of planktonic bacteria.

Single-species biofilms have been thoroughly studied in the majority of reductionist investigations of biofilm biology. However, biofilms in nature are typically composed of multiple species, where interspecies interactions can affect how these communities form, are structured, and work in contrast to biofilm populations. To investigate how interspecies interactions influence biofilm development, structure, and stress responses, a reproducible mixed-species biofilm made up of *Pseudomonas aeruginosa, Pseudomonas protegens,* and *Klebsiella pneumoniae* was modified. To identify each species' abundance and geographic localization within the biofilm, each was fluorescently tagged. Different structures in the mixed-species biofilm stood out from those in similar single-species biofilms. Additionally, compared to single-species biofilms, the formation of the mixed-species biofilm took 1-2

days longer. Along the flow cell canal, where nutrient conditions and each species' growth rate may have an impact on community assembly, the composition and spatial structure of the mixed-species biofilm also changed. Strangely, compared to single-species biofilms, the mixed-species biofilms were more immune to the antimicrobials sodium dodecyl sulfate and tobramycin. Importantly, it was discovered that such community resilience was not the result of selection for the resistant species but rather a security provided to the entire community by the resistant species. In comparison, mixed-species planktonic cultures did not exhibit community-level resilience. These results indicate that structured biofilm communities, where members are tightly entwined, are the only ones to engage in community-level interactions like sharing of common goods [8].

CONCLUSION

Given that pure planktonic growth is rare, the generalized notion that bacteria have a unicellular way of life is not completely accurate. An ordered community of microorganisms called a biofilm is described as existing within a self-produced matrix of polymeric materials that adhere to various surfaces.

This review's goals were to provide an overview of the mechanism underlying biofilm formation, as well as to emphasize its consequences for both human and animal health and available control measures. Five stages are involved in the development of a biofilm: cell attachment, secure affixing of cells, formation of microcolonies and their early stages of maturation, further maturation, and cell dispersal from the biofilm. The type of surface, the characteristics of the medium, and the characteristics of the microbial cell surface are all factors that affect cell adhesion.

The interaction between environmental cues and the microorganisms' reciprocation of the associated signaling events results in the formation of a biofilm. Microbiological organisms and EPS make up the majority of biofilms.

The role of biofilm in the development of disease in both humans and animals is now generally acknowledged. The risk of infection is likely higher in animal species than in people. Medical device-related infections in humans included those caused by pacemakers, electrical dialyzers, joint prostheses, intravenous catheters, and urinary catheters. Food processing equipment can be a constant source of spoilage and pathogenic bacteria if microorganisms develop biofilm on it, as food is known to be a very effective way to expose a large number of people to a possible hazard. In a perfect world, avoiding biofilm formation would be preferable to treating it. Cleaning and disinfecting frequently is the primary method of preventing biofilm formation before bacteria become securely attached to surfaces. More than 90% of the surface-associated microorganisms can be eliminated by this procedure. In recent years, the use of bacteriophages and acid shock therapy has been investigated. Even though there are control methods, prevention is the best course of action because it is more challenging to prevent and control the formation of biofilms. A particular emphasis needs to be placed on the creation of better control and prevention techniques with better results.

REFERENCES:

[1] M. Crouzet *et al.*, “Exploring early steps in biofilm formation: Set-up of an experimental system for molecular studies,” *BMC Microbiol.*, 2014, doi: 10.1186/s12866-014-0253-z.

[2] “Review on biofilm formation and its control options,” *Int. J. Adv. Res. Biol. Sci.*, 2017, doi: 10.22192/ijarbs.2017.04.08.017.

[3] S. Moorthy and P. I. Watnick, "Genetic evidence that the Vibrio cholerae monolayer is a distinct stage in biofilm development," *Mol. Microbiol.*, 2004, doi: 10.1111/j.1365-2958.2004.04000.x.

[4] B. M. Prüß, C. Besemann, A. Denton, and A. J. Wolfe, "A complex transcription network controls the early stages of biofilm development by Escherichia coli," *Journal of Bacteriology*. 2006. doi: 10.1128/JB.01780-05.

[5] C. Ryder, M. Byrd, and D. J. Wozniak, "Role of polysaccharides in Pseudomonas aeruginosa biofilm development," *Current Opinion in Microbiology*. 2007. doi: 10.1016/j.mib.2007.09.010.

[6] G. A. O'Toole and R. Kolter, "Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development," *Mol. Microbiol.*, 1998, doi: 10.1046/j.1365-2958.1998.01062.x.

[7] M. A. S. Alem, M. D. Y. Oteef, T. H. Flowers, and L. J. Douglas, "Production of tyrosol by Candida albicans biofilms and its role in quorum sensing and biofilm development," *Eukaryot. Cell*, 2006, doi: 10.1128/EC.00219-06.

[8] K. W. K. Lee, S. Periasamy, M. Mukherjee, C. Xie, S. Kjelleberg, and S. A. Rice, "Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm," *ISME J.*, 2014, doi: 10.1038/ismej.2013.194.

CHAPTER 12

MAJOR COMPONENTS INVOLVED IN THE BIOFILMS FORMATION

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ABSTRACT:

A self-produced matrix of hydrated extracellular polymeric substances (EPS) serves as the immediate habitat for the microorganisms in biofilms. Polysaccharides, proteins, nucleic acids, and lipids make up the majority of EPS, which gives biofilms their mechanical stability, facilitates their adhesion to surfaces, and forms a cohesive, three-dimensional polymer network that links and momentarily immobilizes biofilm cells. Additionally, by keeping extracellular enzymes close to the cells, the biofilm matrix functions as an external digestive system, allowing the cells to metabolize dissolved, colloidal, and solid biopolymers. Here, we discuss the features, characteristics, and EPS matrix components that make biofilms the most resilient types of life on Earth.

KEYWORDS:

Bacterial Life, Biofilm Matrix, Extracellular Matrix, Extracellular DNA, Matrix Compound.

INTRODUCTION

Biofilms are bacterial populations contained in a matrix that are attached, surfaces, and/or interfaces. They are primarily made of polysaccharides, proteins, lipids, and extracellular DNA. By interacting with a surface and beginning to create an extracellular matrix that holds them together and binds them to it, the cells change from a motile to a sessile lifestyle during biofilm formation. Sessile cells, in contrast to their non-encased, free-swimming counterparts, the planktonic cells, are the cells that create biofilms. Recent research suggests that biofilms, which predominate in every habitat on earth, are the primary source of active bacterial life. The biofilm lifestyle offers the integrating cells several advantages over the planktonic lifestyle, including protection from antimicrobial agents and predators, tolerance for shifting environmental circumstances, and colonization aptitudes [1].

The creation of an extracellular polymeric biofilm structure is a defining characteristic of biofilms. The ability of bacteria to produce an extracellular substance that facilitates attachment was first identified in the pre-molecular era by when fouling organisms are in the planktonic or free-swimming stage, they may create a mucilaginous surface to which they easily adhere until they can create their holdfast. It is now understood that the biofilm matrix created by the majority of organisms typically consists of external proteins, lipids, exopolysaccharides, and eDNA, many of which have characteristics similar to those of amyloid. The remarkable ability of biofilm communities to support the growth and survival of cells in their immediate surroundings is dependent on the production of the biofilm matrix.

The extracellular matrix plays a variety of roles within the biofilm, and as a result, it has a variable makeup among various microbial species. One method used by various bacterial species to give the biofilm morphological integrity/rigidity is the production of protein fibers that serve as a scaffold for the attachment of cells and other matrix elements, such as exopolysaccharides. For the residents, other matrix elements serve a protective purpose. For

instance, the cellulose produced by Escherichia coli biofilms improves the community's resistance to, and the bacterial hydrophobin BslA forms a water-resistant "raincoat" over the Bacillus subtilis biofilm. Further matrix components facilitate interactions between bacteria and host cells: for example, while curli fibers produced by E. coli form a structural component of the biofilm, they are also required for the attachment of the E. coli cells to a variety of protein components of the host cells at the onset of infection. Controlling the initiation, stabilization, or dispersion of biofilms requires a thorough knowledge of the molecular function of such components [2].

Extracellular proteins, which make up a large portion of the biofilm matrix, have gotten less research than other components like EPSs (Figure.1). The biofilm matrix contains proteins that serve structural and medical purposes. Some matrix proteins have extracellular enzyme properties and are linked to processes like the recycling and degradation of biopolymers for nutrient availability as well as the alteration of other exopolymers for cell shaping or cell release from the biofilm structure. Lipases, hydrolases, lyases, and glycanases are some of the enzymes that function in the biofilm matrix in this way. Although this function has not been examined in the context of biofilms on plant surfaces, some enzymes released by pathogenic bacteria may serve as virulence factors.

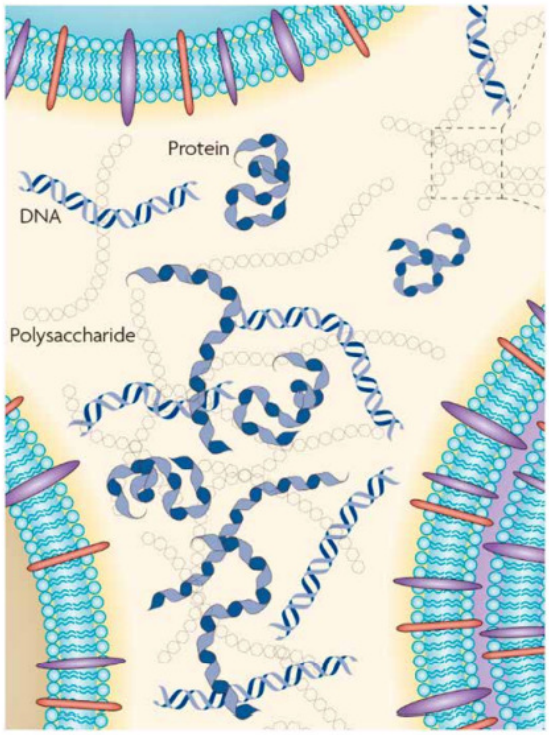

Figure 1: Biofilms matrix: Showing the distribution of the major extracellular components (christeyens).

In the biofilm matrix, some proteins serve structural purposes, such as acting as lectins to attach bacterial cells to the polymeric matrix. A glucan-binding protein in *Streptococcus mutans*, LecA and LecB in *P. aeruginosa*, TasA in *Bacillus subtilis*, and lectins in *A. brasilense* are a few examples of these external carbohydrate-binding proteins (Figure.2). Outer membrane vesicles, a typical component of the matrix biofilm in this species, were discovered to contain a significant amount of matrix proteins in *P. aeruginosa*. Another typical class of matrix protein with extracellular adhesin activity is amyloids.

Bacterial species	Name of protein	Experimental evidence of amyloid properties	Function within biofilm
Bacillus subtilis	TasA (TapA minor component)	Electron and atomic force microscopy; Thioflavin T and Congo red binding propensity; CD spectrum profile	Biofilm matrix component
Enterobacter cloacae	Curli (CsgA)	Similarity at gene level to *csg* operon; electron microscopy of whole cells	Biofilm matrix component
Escherichia coli	Curli (CsgA)	Electron microscopy; Thioflavin T and congo red binding propensity; CD spectrum profile; NMR	Biofilm matrix component; adhesion
Pseudomonas spp.	Functional amyloid pseudomonas (Fap) fimbriae (FapC with FapB as a minor element)	Electron microscopy; CD spectrum profile; highly stable protein fibres	Biofilm matrix component
Salmonella ssp.	Curli (alternatively Tafi for thin aggregative fimbrae) (CsgA)	Electron microscopy; highly stable protein fibres	Biofilm matrix component; resistance to antibacterial agents; adhesion to surfaces
Staphylococcus aureus	Phenol Soluble Modulins	Electron microscopy; Thioflavin T binding propensity; detergent resistant fibre	Biofilm matrix component; biofilm stability; amyloid formation blocks dispersal activity of monomeric PSM *in vitro* under specific growth conditions; Not identified to date if synthesized *in vivo*
Streptococcus mutans	Cell surface localized antigen P1 (PAc)	Electron microscopy; Thioflavin T and congo red binding propensity; detergent-resistant protein fibres	Biofilm matrix component

Figure 2: Biofilms protein: Showing the overview of the different proteins involved in the biofilms formation (Semantic scholar).

A perfect environment for the sharing of genetic material is provided by biofilms. Bacterial communities in biofilms have been shown to conjugate at higher rates than planktonic bacteria. eDNA is a crucial component of the biofilm matrix and aids in the development of biofilms in several bacterial species, such as *P. aeruginosa* and *Bacillus cereus*. According to the bacterial genus, eDNA varies in size, location, and origin. According to some research, programmed cell death may occur in biofilms because of how eDNA is arranged and how its release is dependent on the lysis of specific types of bacteria. eDNA plays a role in bacterial autoaggregation and attachment to hydrophobic surfaces in Gram-positive bacteria.

Lipids are part of the biofilm matrix as well, even though research on plant-bacterial interactions hasn't focused much on them. In biofilms, lipids typically serve as biosurfactants with a variety of roles, including surface activity, hydrophobic substance dispersal and bioavailability, antibacterial or antifungal characteristics, and bacterial attachment and detachment. These characteristics of *P. aeruginosa* rhamnolipids have been well characterized, and they are crucial for surface interaction, microcolony formation, structural maintenance, and biofilm dispersal, among other phases of biofilm development. Extracellular proteins, eDNA, and lipids in the biofilm matrix of plant-associated bacteria are still poorly understood in terms of their identities and roles. Our knowledge of the process of biofilm formation will be significantly improved by additional research in this area [3].

LITERATURE REVIEW

Microbe colonies that are attached to surfaces and enclosed by an extracellular matrix are called biofilms. Although the creation of biofilms is thought to be involved in 80% of all bacterial infections, little is known about their composition and control. The function of extracellular DNA (eDNA), a significant structural component in many Staphylococcus aureus biofilms, is unclear. Here, we demonstrate that beta toxin, a neutral sphingomyelinase and a virulence factor of S. aureus, forms covalent cross-links to itself in the presence of DNA (we refer to this as biofilm ligase activity, independent of sphingomyelinase activity) producing an insoluble nucleoprotein matrix in vitro. Furthermore, by implicating beta toxin in the development of infectious endocarditis in a rabbit model, we establish that the toxin strongly stimulates biofilm formation in vivo. These findings collectively imply that beta toxin cross-linking in the presence of eDNA aids in the formation of the structural foundation for staphylococcal biofilms [4].

In recent literature, DNA has been identified as a key structural element of the extracellular matrix in biofilms. The competence-stimulating peptide (CSP) cell-to-cell signal is implicated in streptococci's ability to undergo a genetic transformation, form biofilms, and undergo autolysis. The genes involved in binding and ingesting extracellular DNA are among those regulated in reaction to the CSP. In this research, we demonstrate that a functional DNA binding-uptake system contributes to the formation of biofilms. Reduced biofilm development was observed in a *Streptococcus mutans* comGB mutant that was defective in DNA binding and uptake but not in signaling. During growth in the presence of DNase I, biofilm was reduced in the wild-type to levels comparable to those found with the comGB mutant, indicating that DNA plays an essential role in the wild-type biofilm formation. We also demonstrated that the amounts of DNA released during growth in the presence of synthetic CSP were comparable between the comGB mutant and the wild type. The significance of the DNA binding-uptake pathway in the development of biofilms suggests potential new targets for the treatment of infections [5].

Structured bacterial colonies known as biofilms are attached to a surface and enclosed in a self-made matrix of extracellular polymeric materials. Due to their high resistance to antimicrobial agents, biofilms are at the root of a wide variety of issues, including quality and safety concerns in the food business. Recently, major advances have been made in elucidating the different structural components of the biofilm matrix, the regulatory pathways involved in biofilm formation, and signaling molecules involved in biofilm formation and dispersal, which provide opportunities for the prevention and control of these biofilms in the food industry [6].

The biofilm matrix is a dynamic habitat where the constituent microbial cells seem to achieve homeostasis and are best arranged to utilize all nutrients available. Microbial cells, polysaccharides, water, and excreted biological products are the main matrix constituents. As a result, the grid exhibits significant microheterogeneity, allowing for the existence of numerous microenvironments. Although exopolysaccharides provide the matrix framework, the biofilm contains a broad variety of enzyme activities, some of which have a significant impact on structural integrity and stability [7].

Pseudomonas aeruginosa creates various biofilms, or matrix-enclosed, surface-associated multicellular formations that help it survive in a range of conditions. The pellicle that develops at the air-liquid contact in standing cultures is one example of a biofilm. We looked for *P. aeruginosa* PA14 transposon insertion mutants that could not produce pellicles. Seven adjacent genes, known as pel genes, whose products appear to be involved in the creation of

the pellicle's extracellular matrix, were discovered through analysis of these mutants. The pel genes are necessary for the development of solid surface-associated biofilms in addition to pellicle production. Sequence analyses indicated that five pel genes have functional homologs involved in carbohydrate processing and that three pel genes encode transmembrane proteins. Microscopic and macroscopic observations showed that the pel mutants don't create any extracellular matrix, while the wild-type *P. aeruginosa* PA14 produces a cellulase-sensitive extracellular matrix that can bind Congo red. Compared to the carbohydrates generated by the pel mutants and the wild-type strain, glucose appeared to be the main component of the matrix material. Together, these findings imply that the pel genes are in charge of creating the glucose-rich matrix material needed by *P. aeruginosa* PA14 to create biofilms [8].

Due to the spread of antibiotic-resistant strains, Staphylococcus aureus and *Staphylococcus epidermidis* are two major human pathogens of rising significance. Evidence points to the pathogenesis of *S. aureus* and *S. epidermidis* being aided by their capacity to create matrix-encased biofilms. In this research, we examined the roles of two staphylococcal biofilm matrix polymers: extracellular DNA and poly-N-acetylglucosamine surface polysaccharide (PNAG). (ecDNA). In a 96-well microtiter plate experiment, we evaluated the capacity of a PNAG-degrading enzyme (dispersin B) and DNase I to prevent the growth of new biofilms, separate existing ones, and make biofilms more susceptible to destruction by the cationic detergent cetylpyridinium chloride (CPC). Dispersion B and DNase I both prevented *S. aureus* and *S. epidermidis* from forming biofilms when introduced to the growth medium. Dispersin B and DNase I both remove preformed S. aureus and *S. epidermidis* biofilms, but not preformed S. aureus and S. aureus biofilms. Similar to how DNase I sensitized S. aureus biofilms to CPC death, dispersin B sensitized *S. epidermidis* biofilms did the opposite. We concluded that the structural roles that PNAG and ecDNA play in *S. aureus* and *S. epidermidis* biofilms are essentially different [9].

Biofilms, which are cellular aggregations enclosed in an extracellular matrix, are formed by *Pseudomonas aeruginosa*. Two loci, pel and psl, which are involved in the production of carbohydrate-rich components of the biofilm matrix, were discovered through molecular genetic studies of three prevalent autoaggregation phenotypes, including wrinkled colonies, pellicles, and solid-surface-associated biofilms. In *P. aeruginosa* strain PA14, the pel gene cluster is implicated in the synthesis of the glucose-rich matrix material. Here, we examine the function of the pel gene cluster in *P. aeruginosa* strain ZK2870 and locate a second genomic region called psl that is involved in the synthesis of a mannose-rich matrix substance. Proteins involved in the processing of carbohydrates are homologous to the 11 expected protein products of the psl genes. As a result, *P. aeruginosa* can create two different carbohydrate-rich matrix materials. Both carbohydrate-rich matrix components seem to be necessary for the development of mature biofilms in *P. aeruginosa* isolates PA14 and ZK2870, and at least one of them is necessary[10].

CONCLUSION

In conclusion, the bacterial biofilm matrix is a highly complicated environment that enables the hosted cells to live very differently from planktonic cells. Exopolymer substances (EPS) are necessary for the development of biofilms and their variety offers a variety of functions that allow the survival and growth of the biofilm's cells against external aggressions. Because of the complexity of the biofilm matrix and the degree of protection offered to microorganisms by this form of life, the formation of biofilms presents a serious threat to food safety, and their control necessitates the use of specialized equipment and the application of specific hygiene procedures.

REFERENCES

[1] T. Rasamiravaka, Q. Labtani, P. Duez, and andMondher El Jaziri, "The Formation of Biofilms by," *Biomed Res. Int.*, 2015.

[2] L. Hobley, C. Harkins, C. E. MacPhee, and N. R. Stanley-Wall, "Giving structure to the biofilm matrix: An overview of individual strategies and emerging common themes," *FEMS Microbiology Reviews*. 2015. doi: 10.1093/femsre/fuv015.

[3] P. C. Bogino, M. de las M. Oliva, F. G. Sorroche, and W. Giordano, "The role of bacterial biofilms and surface components in plant-bacterial associations," *International Journal of Molecular Sciences*. 2013. doi: 10.3390/ijms140815838.

[4] M. J. Huseby *et al.*, "Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms," *Proc. Natl. Acad. Sci. U. S. A.*, 2010, doi: 10.1073/pnas.0911032107.

[5] F. C. Petersen, L. Tao, and A. A. Scheie, "DNA binding-uptake system: A link between cell-to-cell communication and biofilm formation," *J. Bacteriol.*, 2005, doi: 10.1128/JB.187.13.4392-4400.2005.

[6] T. Abee, Á. T. Kovács, O. P. Kuipers, and S. van der Veen, "Biofilm formation and dispersal in Gram-positive bacteria," *Current Opinion in Biotechnology*. 2011. doi: 10.1016/j.copbio.2010.10.016.

[7] I. W. Sutherland, "The biofilm matrix - An immobilized but dynamic microbial environment," *Trends in Microbiology*. 2001. doi: 10.1016/S0966-842X(01)02012-1.

[8] L. Friedman and R. Kolter, "Genes involved in matrix formation in Pseudomonas aeruginosa PA14 biofilms," *Mol. Microbiol.*, 2004, doi: 10.1046/j.1365-2958.2003.03877.x.

[9] E. A. Izano, M. A. Amarante, W. B. Kher, and J. B. Kaplan, "Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms," *Appl. Environ. Microbiol.*, 2008, doi: 10.1128/AEM.02073-07.

[10] L. Friedman and R. Kolter, "Two genetic loci produce distinct carbohydrate-rich structural components of the Pseudomonas aeruginosa biofilm matrix," *J. Bacteriol.*, 2004, doi: 10.1128/JB.186.14.4457-4465.2004.

CHAPTER 13

BACTERIAL COMMUNITIES SUCCESSION IN ENVIRONMENTAL CONDITIONS PRESENT IN THE BIOFILMS

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ABSTRACT:

Bacterial biofilms, the primary form of bacterial life in nature, are made up of various bacterial species that come together to create a dynamic and complex community. Their biological importance is derived from their effects on ecology, pathology, agriculture, production, and remediation, among other things. Their development is marked by profound changes at both the taxonomic and physiological levels. Most research uses streamlined lab-scale models to concentrate on the subjects' structural and physiological features. The foundation and succession of bacterial communities assembled as biofilm-like structures in biotic and abiotic surfaces, or even in natural environments, are thus poorly understood. These communities have recently been given a more in-depth look thanks to molecular and bioinformatics tools, and the taxonomic changes within them are starting to be examined in terms of species succession. Initially, succession at the ecological level defined the patterns of establishment and change of superior organisms within a specific ecosystem. Through various models, these concepts have been modified to conceptualize the succession of microbial communities. Therefore, bacterial succession in biofilms has become a novel and important aspect to comprehend their operation. By discussing knowledge collected from various environments, this chapter provides an overview of the key advancements in the field.

KEYWORDS:

Bacterial Community, Biofilm Structure, Community Structure, Microbial Communities, Temporal Succession.

INTRODUCTION

An assembly of microbial cells adhered to a surface and enclosed in a self-produced extracellular polymeric matrix is referred to as a biofilm. Through successional processes, planktonic and motile bacteria move from the top to an aggregating biofilm. Adsorption of dissolved organic molecules and primary colonization of free-living bacteria on the surface triggers the accumulation of bacteria through growth and reproduction, which modifies the characteristics of the surface and renders it suitable for subsequent colonization by secondary microorganisms. The initial colonizer and planktonic bacteria interact specifically and/or inadvertently to create the primary biofilm community, and distinct pioneer microorganisms contribute to biofilm formation in various environments. Biofilm maturation occurs through interactions between colonized species that are synergistic and/or competitive, as well as through the recruitment of new species and/or eradication of colonized species [1].

By improving access to nutrients, allowing cometabolic interactions with nearby microorganisms, and protecting against toxins and antibiotics, biofilms are a protective mode of growth that enables microorganisms to live in hostile or oligotrophic environments. Additionally, biofilms are essential for primary production, the biodegradation of organic matter and contaminants, and the recycling of nutrients in the natural world. However, in

aquatic settings, biofilms can be harmful to the surfaces of man-made structures like ships and bridges. Numerous studies have been conducted on the bacterial communities, developmental processes, and physiology of biofilms in different aquatic environments as a result of the significant role that biofilms play in ecology and industry. Additionally, techniques to control biofilm formation have been created [1].

The distance from the retreating glacier is used as a stand-in for soil age because a glacier Chrono sequence is defined by a collection of locations with the same parent material and substrates. Under this vision, the mineral soil closer to the glacier terminus is usually vegetation free and heterogeneously composed of distinct geological and pedological morphotypes, i.e., recent sandy deposits, exposed rock materials, erosion channels, floodplains, and mudslides with low amounts of carbon, nitrogen, and other nutrients. Plant establishment used to be regarded as the first stage of the main succession until a few years ago. The ability of a diverse microbial community to colonize recently exposed substrates long before lichens, non-vascular plants, and vascular plants are now well documented. Consequently, the creation of fertile soil where complex vegetation communities can grow and evolve is a cascade of processes that begins with microbial colonization. (Figure. 1).

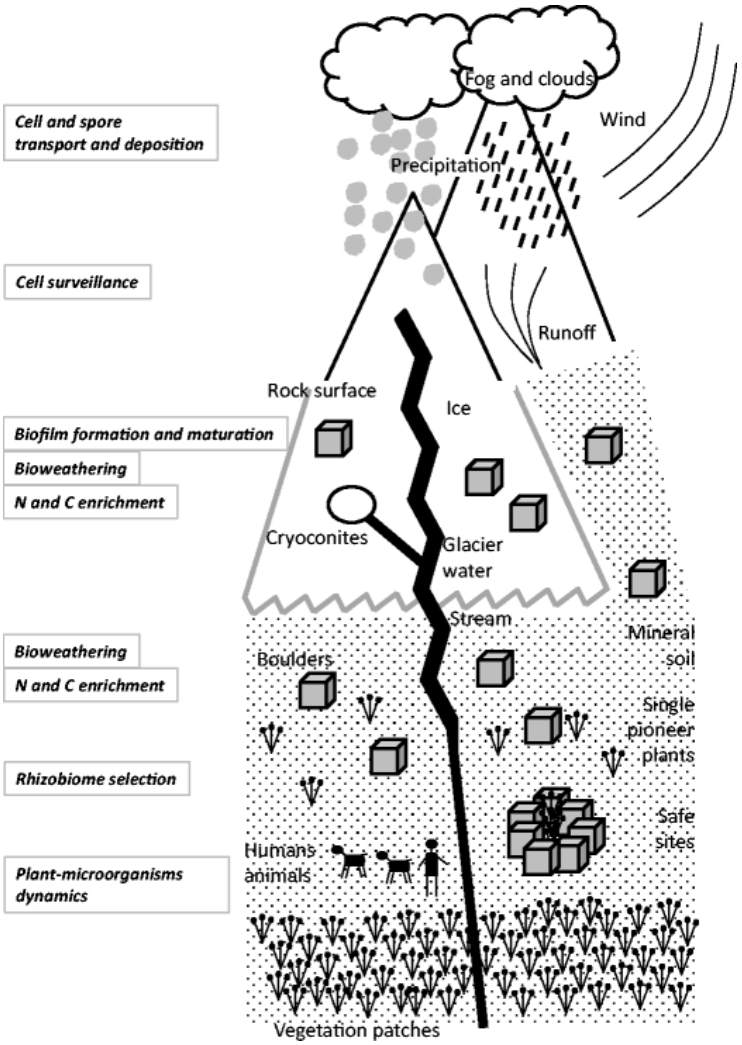

Figure. 1: Microbial community: Diagramed showing the microbial community and the primary succession process in the hill area (annalsmicrobiology).

Therefore, essential and basic actors capable of enriching mineral soil with nitrogen and carbon include bacteria, archaea, fungi, and algae. On the other hand, the opposing processes

of denitrification, anammox, methanogenesis, and microbial respiration result in the loss of organic matter and minerals. Through their ecological behavior and developmental strategies, microorganisms in this complex equilibrium must constantly respond to habitat change, inter-kingdom, and trans-kingdom intra-species competition. Reviewing primary succession processes in high mountain settings in temperate areas from the perspective of microbial communities in this paper is important because it highlights the role that these communities played in the colonization of mineral soil and pioneer plants (Figure. 1)

It is now known that bacterial communities that have colonized submerged substrata are a major contributor to the complicated biofouling phenomenon that occurs in the marine environment. Studies documenting pioneer bacterial colonizers and community succession during the early-stage biofilm are few and far between, despite the intense maritime activity and sizable industrial sector in the Laccadive Sea's nearshore. We looked at the biofilm-forming bacterial population succession on three different substrates stainless steel, high-density polyethylene, and titanium over 15 days of immersion in a power plant's seawater intake area in southern India. Illumina MiSeq sequenced 16S rRNA gene amplicons were used to examine the bacterial community makeup of biofilms and nearby seawater.

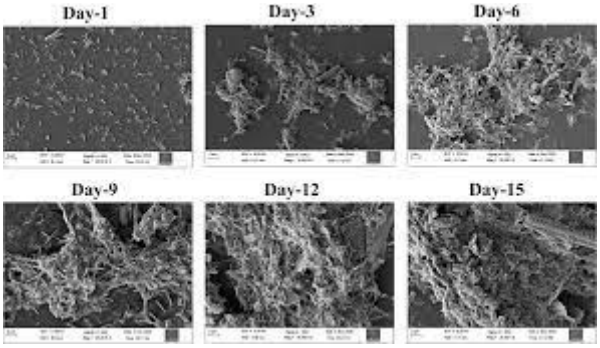

Figure 2: Bacterial commutes: Diagramed showing the different succession phases of the bacterial (PLOS).

The obtained metataxonomic findings showed a significant impact of substrate type over temporal succession on the early-stage biofilm-forming microbiota. Bacterial communities displayed striking temporal dynamics with changes in numerous bacterial families. Over the biofilm succession days, the proportion of the dominant phyla, Proteobacteria, declined while Bacteroidetes increased, indicating their roles as early and late colonizers, respectively. Throughout the successional phases, a sharp fluctuation in the relative abundance of the two bacterial orders Alteromonadales and Vibrionales was seen. While no substrata type-specific bacterial groups were found, LEfSe analysis did identify particular bacterial groups at all stages of biofilm development. Additionally, it was shown by the findings of PCoA and UPGMA hierarchical clustering that the biofilm-forming community was very different from the planktonic community (Figure.2). The planktonic community was led by the Bacteroidetes, Cyanobacteria, and Actinobacteria, while the biofilm-forming community was dominated by the phylum Proteobacteria. Overall, our findings show that the temporal succession overshadowed the effect of the substrate material, contradicting the widely held belief that the substrate material has a significant influence on biofilm formation [2].

LITERATURE REVIEW

It is well known that marine biofilms can affect how metal surfaces corrode in the maritime environment. Despite some new studies, some temporal settings of the succession of bacterial communities colonizing artificial surfaces remain uncharacterized. More specifically, it is

unclear whether bacteria that colonize artificial surfaces are comparable or different depending on the time of year. The research of early biofilms, in which bacterial cell communities initially adhere to artificial surfaces, is particularly important for the growth of later biofilm communities. In this study, we used universal 16S rRNA bacterial primers and amplicon-based NGS (next-generation sequencing) to describe the early biofilm bacterial communities developing on 316 L stainless steel surfaces in a Northern Portuguese port. Sampling took place over two separate 30-day seasons. (spring and winter). Planktonic communities from the same area and biofilm communities developing on steel surfaces painted with an anti-corrosion paint were also studied. Our findings showed that the sampled seasons showed unique temporal patterns. Particularly, a higher abundance of Alphaproteobacteria was discovered during the same days of biofilm growth in winter, and a higher abundance of Gammaproteobacteria and Mollicutes was discovered on the first days of biofilm development in spring (day 1 to day 4). The spring biofilms notably changed toward dominance of photoautotrophic groups (mostly diatoms) on the final sampling day (day 30), and some macrofouling communities colonized them as well, which was not seen during the winter sampling. Our findings showed that, rather than the general impact of the season or the overall sampling day of both seasons, the sampled day of the particular season had a greater impact on the bacterial composition in the biofilms. A non-fouling-release anti-corrosion paint was also applied to the steel plates, but this only caused a noticeably reduced diversity when compared to plates without paint. This difference was only seen in the spring. We recommend that future antifouling/anti-biofilm uses take into account the temporal succession of marine biofilm communities [3].

To ensure that safe, high-quality water reaches users after passing through these large-surface-area reactors, it is crucial to comprehend the temporal dynamics of multi-species biofilms in drinking water distribution systems (DWDS). This study examined the successional traits of bacterial and fungal populations in artificial environments that were perfectly resemblant to real-world DWDS. After one month of biofilm growth, microbial communities were seen to become more complex, but they did not become stable after three months. Despite ongoing changes in the makeup of the bacterial population, changes in cell numbers were more rapid at the beginning of biofilm formation and tended to decline over time. In comparison to bacterial diversity, fungal diversity was significantly lower and lagged in its response to time dynamics. The bacteria *Pseudomonas*, *Massillia*, and *Sphingomonas* as well as the fungi *Acremonium* and *Neocosmopora* were reliably present in the biofilms throughout the time and conditions examined. The existing aging DWDS infrastructure must be monitored and managed to ensure the delivery of safe drinking water[4]. This includes managing biofilms and other pervasive core microbial communities.

One of the primary causes of membrane biofouling in membrane bioreactors is the development of biofilms. (MBRs). As a result, it's critical to pinpoint the organisms at fault when creating focused biofouling control methods. To correlate these changes with an increase in transmembrane pressure, this research analyzed the composition and alterations in the microbial communities fouling MBR membranes over time. (TMP). According to qPCR data, bacteria accounted for 92.9–98.4% of the biofilm's species, outweighing fungi (1.5–6.9%) and archaea (0.03-0.07%). According to NMDS analysis, the biofilm communities were identical to those found in the sludge in the early phases of operation. But over time, the biofilm community considerably separated from the sludge and eventually displayed a distinct biofilm profile. This indicated that a population of organisms that were experts at growing biofilms had undergone strong selection. The rapid rise in TMP, where bacteria like *Rhodospirillales, Sphingomonadales,* and *Rhizobiales* predominated the biofilm at the time, was linked with this successional and selective pattern. The majority of the discovered fungal

OTUs matched Candida species, but 18S rRNA gene sequencing failed to classify the majority of the fungal communities. Collectively, the results imply that fungi and bacteria, in particular, may play a significant role in the rapid rise in TMP and decline in system performance [5].

Around the globe, artificial reefs (ARs) are frequently made of concrete and wood and are used to improve marine resources and restore habitat. Although microbial biofilms are crucial to marine environments, little is known about the microbial communities that inhabit concrete and wooden ARs and their temporal succession. This research looked into the factors that influenced the temporal succession of the microbial communities on concrete and wooden AR blocks. The relative abundances of Proteobacteria, Cyanobacteria, and Gracilibacteria among the six dominant phyla significantly declined in wood and concrete, respectively, as did those of Cyanobacteria, indicating that the composition of the microbial communities changed over time. In comparison to wood, concrete had considerably higher OTU richness and Shannon indices. The microbial communities were organized into two distinct groups that corresponded to the two substrate materials using non-metric multidimensional scaling ordination. Concrete and wood both had macrobenthic compositions that were largely comparable and changed over time, particularly in the first five weeks. With the organism coverage, the Shannon index of the microbial communities in concrete and wood greatly increased. The findings add to our knowledge of the ecological effects of ARs[6] and provide fundamental information on microbial community succession during the initial deployment of ARs.

Previous research has demonstrated that the polychaete Hydroides elegans' larval settlement can be mediated by biofilms and that variations in the density and composition of biofilms frequently affect the larval settlement response. The chemical cues that cause this reaction are still unknown, though. The chemical profiles of subtidal biofilms and successional changes in the bacterial community structure are both described in this research and linked to the response of larvae to settlement. Over the course of 20 days, multispecies biofilms formed on a granite rock and polystyrene Petri plates in the subtidal zone. Two molecular techniques (microarray (PhyloChip) and denaturing gradient gel electrophoresis) and gas chromatography-mass spectrometry, respectively, were used to assess the impacts of the substrate and age on the bacterial community structure and chemical profiles of the biofilms. The bacterial community patterns and chemical profiles of the biofilms were modified by both age and substrate. The substratum had less of an impact on the structure of the bacterial population than age did. The chemical profile, however, was more significantly impacted by the sort of substrate. Extracts from biofilms that had formed over time and on various substrates had been examined for *H. elegans* larval settlement. The larval settlement was induced by the extracts in a biofilm age-dependent way, and there was no difference in a larval settlement between extracts derived from various substrata of the same age. Our findings imply that the biofilm's overall chemical makeup alone cannot forecast the larval settlement response [7].

Using 16S rRNA gene-based polymerase chain reaction-denaturing gradient gel electrophoresis (DGGE) and sequence analysis, temporal bacterial population changes in river biofilms were examined. In the River Garonne, naturally occurring biofilms were collected in 2001 during an unaltered seven-month low-water phase. (SW France). Epilithic biomass showed a distinct pattern during the sampling period: two 3-month periods of accumulation led to two peaks in the summer and autumn, each at about 25 g ash-free dry mass per square meter. Indicating the impact of seasonal factors on these communities, the DGGE profiles of the bacterial communities varied between the summer and autumn biomass

peaks and only shared 30% of the operational taxonomic units (OTUs). Bacterial diversity and the emergence of fresh OTUs during the second biomass accrual period were consistent with a theoretical model of bacterial biofilm succession.

Five OTUs (corresponding to *Dechloromonas sp*., *Nitrospira sp*., and three different Spirosoma spp.) displayed specific patterns during succession and were only present during clearly defined successional stages, indicating that epilithic bacteria have different life-history strategies. The co-inertia analysis of DGGE banding patterns and physical-chemical data revealed a significant relationship between community structure and environmental factors, indicating that hydrodynamic stability and seasonal changes in temperature and light were the primary factors influencing bacterial communities. Analysis of environmental factors and community patterns during the stable periods revealed that time and maturation had a dominant impact on the structure of the bacterial community. As a result, succession in these groups of epilithic biofilms that are found in nature seems to be influenced by both allogenic (seasonal) and autogenic changes [8].

It has been demonstrated that the drinking water sanitizer monochloramine increases the levels of mycobacteria and nitrifying bacteria. In a water distribution system simulator, 16S rRNA gene clone libraries made from various biofilms were used to look into the possible successions and development of these bacteria. Using borosilicate glass beads, and polycarbonate coupons from annular reactors incubated for up to 8 months in water treated with monochloramine, and in-line and off-line devices, biofilms were formed. In terms of community structures, there were no appreciable differences between biofilm devices and coupon material, but all biofilm communities that formed on various devices experienced comparable successions over time. *Serratia* (29%), *Cloacibacterium* (23%), *Diaphorobacter* (16%), and *Pseudomonas* (7%), which predominated in the early phases of biofilm formation, were followed by Mycobacterium-like phylotypes (> 27%) in the later months. Individuals with predisposing conditions may be affected by the development of nontuberculous mycobacteria (NTM) after three months, while nitrifiers (such as *Nitrospira moscoviensis* and *Nitrosospira multiformis*) may affect water quality. Overall, the phyla Proteobacteria, Actinobacteria, and Bacteroidetes accounted for 90% of the diversity in all the clone collection samples. These findings offer an ecological perspective on the biofilm bacterial successions in potable water treated with monochloramine[9].

CONCLUSION

In the rhizosphere, the region of soil closest to plant roots, several processes between plants and microorganisms are of critical ecological, interactive, and productive significance. The foundation, succession, and functions of bacterial communities in the rhizosphere, which are reliant on the emergence of biofilm structures, are, however, poorly understood. This research examined alterations in bacterial biofilm communities in the rhizosphere of alfalfa using physiological and molecular characterization. By assembling a condensed community with bacterial strains isolated from artificial surfaces under controlled laboratory circumstances, we assessed natural biofilm-like structures and the early success of artificial biofilm-like structures related to rhizospheric soil. The artificial succession assay revealed distinct bacterial counts, biofilm-forming capacities, and community structures at various time points when compared to natural rhizospheric soil. While -proteobacteria and Actinobacteria predominated in naturally formed mature biofilms in the rhizosphere, highly adhesive strains of -proteobacteria dominated the early phases of biofilm formation associated with roots. Short experimentation times in adhesion assays on the roots revealed changes in the structure and dynamics of the bacterial population, which were in good agreement with outcomes on synthetic surfaces. Summing up, the study found that the establishment of multilateral

communities in the rhizosphere of alfalfa is a dynamic process that probably includes the initial formation of biofilm-like structures by highly-adherent strains and the subsequent shaping towards a mature community through mechanisms of replacement and co-existence.

REFERENCES:

[1] Y. M. Lee *et al.*, "Succession of bacterial community structure during the early stage of biofilm development in the Antarctic marine environment," *Korean J. Microbiol.*, 2016, doi: 10.7845/kjm.2016.6005.

[2] T. J. Sushmitha, M. Rajeev, P. S. Murthy, S. Ganesh, S. R. Toleti, and S. K. Pandian, "Bacterial community structure of early-stage biofilms is dictated by temporal succession rather than substrate types in the southern coastal seawater of India," *PLoS One*, 2021, doi: 10.1371/journal.pone.0257961.

[3] J. T. Antunes, A. G. G. Sousa, J. Azevedo, A. Rego, P. N. Leão, and V. Vasconcelos, "Distinct Temporal Succession of Bacterial Communities in Early Marine Biofilms in a Portuguese Atlantic Port," *Front. Microbiol.*, 2020, doi: 10.3389/fmicb.2020.01938.

[4] I. Douterelo, K. E. Fish, and J. B. Boxall, "Succession of bacterial and fungal communities within biofilms of a chlorinated drinking water distribution system," *Water Res.*, vol. 141, pp. 74–85, 2018, doi: 10.1016/j.watres.2018.04.058.

[5] J. Luo, P. Lv, J. Zhang, A. G. Fane, D. McDougald, and S. A. Rice, "Succession of biofilm communities responsible for biofouling of membrane bioreactors (MBRs)," *PLoS One*, vol. 12, no. 7, pp. 1–23, 2017, doi: 10.1371/journal.pone.0179855.

[6] Z. Guo, L. Wang, W. Cong, Z. Jiang, and Z. Liang, "Comparative analysis of the ecological succession of microbial communities on two artificial reef materials," *Microorganisms*, 2021, doi: 10.3390/microorganisms9010120.

[7] H. C. Chung, O. O. Lee, Y. L. Huang, S. Y. Mok, R. Kolter, and P. Y. Qian, "Bacterial community succession and chemical profiles of subtidal biofilms in relation to larval settlement of the polychaete hydroides elegans," *ISME J.*, 2010, doi: 10.1038/ismej.2009.157.

[8] E. Lyautey, C. R. Jackson, J. Cayrou, J. L. Rols, and F. Garabétian, "Bacterial community succession in natural river biofilm assemblages," *Microb. Ecol.*, 2005, doi: 10.1007/s00248-005-5032-9.

[9] R. P. Revetta, V. Gomez-Alvarez, T. L. Gerke, C. Curioso, J. W. Santo Domingo, and N. J. Ashbolt, "Establishment and early succession of bacterial communities in monochloramine-treated drinking water biofilms," *FEMS Microbiol. Ecol.*, 2013, doi: 10.1111/1574-6941.12170.

CHAPTER 14

DIFFERENT FORMS OF BIOFILMS PRESENT IN THE NATURE

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ABSTRACT:

Microorganisms of many different kinds, including bacteria, archaea, protozoa, fungi, and algae, can be found in biofilms; each group has specialized metabolic duties. However, under specific circumstances, some creatures will produce single-species films. A microbial population attached to a surface is called a biofilm. Numerous fungi have the ability to develop biofilms. The formation of biofilm on implanted devices is a key contributor to recurrent infection, making this growth form significant for the biology of infection. Additionally, biofilms are only weakly drug-susceptible, which makes treating device-associated infections very challenging. Even without an implanted device, many infections can develop in a manner akin to a biofilm. Here, we give an overview of our present knowledge of how bacterial, fungal, and algal biofilms are formed, how they are genetically controlled, and how these biofilms develop drug resistance.

KEYWORDS:

Biofilm Microorganisms, Bacterial Biofilms, Biofilm Infection, Extracellular Matrix, Staphylococcus Aureus.

INTRODUCTION

Since Van Leeuwenhoek studied the "animalcules" in the plaque on his teeth in the seventeenth century, biofilms have been described in a variety of systems; however, the general theory of biofilm predominance was not introduced until 1978. According to this hypothesis, the majority of bacteria in all nutrient-sufficient aquatic ecosystems grow in matrix-enclosed biofilms that are attached to surfaces, and these sessile bacterial cells differ significantly from their planktonic (floating) counterparts. The majority of the data used to support this theory came from real aquatic ecosystems, where direct microscopic observations and direct quantitative recovery methods demonstrated that more than 99.9% of the bacteria form biofilms on a variety of surfaces. Except for deep groundwater and abyssal seas, all-natural ecosystems have a predominance of biofilms, and we now understand that these sessile populations are responsible for the majority of physiological processes in these ecosystems. The people who manage industrial water systems were the first to develop techniques to sample sessile bacteria and create strategies to control their expensive depredations because bacterial biofilms cause very severe issues in these systems. The use of biofilm samplers, which are installed into the walls of industrial pipes and vessels, as well as regular testing of the biocides used to protect industrial installations, are now commonplace in industrial systems.

Even though dental plaque is widely recognized as a type of biofilm, the consensus that bacteria primarily grow in matrix-enclosed biofilms in natural and industrial systems was not instantly accepted in the medical and dental fields. The organisms that cause many device-related and other chronic infections, however, actually grow in biofilms in or on these devices, as soon as novel techniques for the direct examination of biofilms became available.

Important intellectual synthesizes started to be produced gradually [1]. Gram-positive and gram-negative bacteria can both create biofilms on medical equipment, but *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans, E. coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa* are the most frequent types. In order to create biofilms, bacteria that adhere to surfaces congregate in a hydrated polymeric matrix of their synthesis (Figure.1). Many persistent and chronic bacterial infections are caused by these sessile communities, which are formed as a result of their natural resilience to antimicrobial agents. Biofilm studies have revealed differentiated, organized cell clusters with community characteristics. Recent developments in our knowledge of the genetic and molecular underpinnings of bacterial community behavior lead to potential therapeutic targets that could offer a method for the management of biofilm infections.

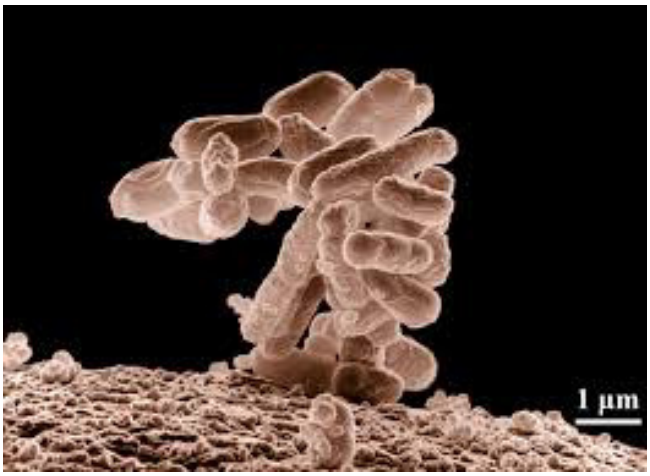

Figure 1: Bacterial biofilms: Diagramed showing the organization of the bacterial biofilms (Prescouter).

A great potential species for research on biofilm formation is filamentous fungi. However, the word "biofilm" is infrequently utilized when discussing As eukaryotic organisms, fungi exhibit peculiar traits like heterotrophic absorption for nutrition, the formation of vegetative and reproductive structures (such as spores and hyphae), and sexual and asexual reproduction. Furthermore, environments with a significant air interface that are subjected to high moisture levels frequently have ff biofilms. (i.e., unsaturated environments). Additionally, by penetrating the substrate on which they develop, frequently exhibit invasive growth. The comprehension of ff biofilm formation and behavior is made more difficult by these variations in morphology and growth. Therefore, drawing inferences from a direct comparison to the dynamics of bacterial biofilms may not be accurate. The capacity of fungi to have more than one planktonic form is one of the distinctive characteristics of fungal biology argues to distinguish fungal biofilm formation from that of bacteria. (i.e., sexual and asexual spores, sporangia, and hyphal fragments) (Figure.2).

The development of specialized reproductive tissues to produce these dispersive forms happens in fungi in response to particular environmental cues, biological stimuli, or stresses. These dispersive forms are not unicellular and frequently float in air rather than water. The aerial component of lifestyle, with a strong reliance on aerial spore dissemination for dispersal in many species, is another intriguing feature of fungal biology that is absent in bacterial biofilms. Small proteins are secreted as part of the fungal aerial development process. (hydrophobins). These microorganisms' growth and evolution are influenced by a number of processes that the hydrophobins, which are unique to, participate in. (formation of

aerial structures, attachment of hyphae to hydrophobic surfaces, and changes in hyphal surface properties in response to environmental and developmental cues). Additionally, it should be kept in mind that fungus hyphae have much larger diameters and lengths than individual bacterial cells. The word "biofilm" is occasionally substituted with terms like "multicellular masses," "pellets," and "submerged/solid-state fermentation" to describe the surface-associated growth of fungi.

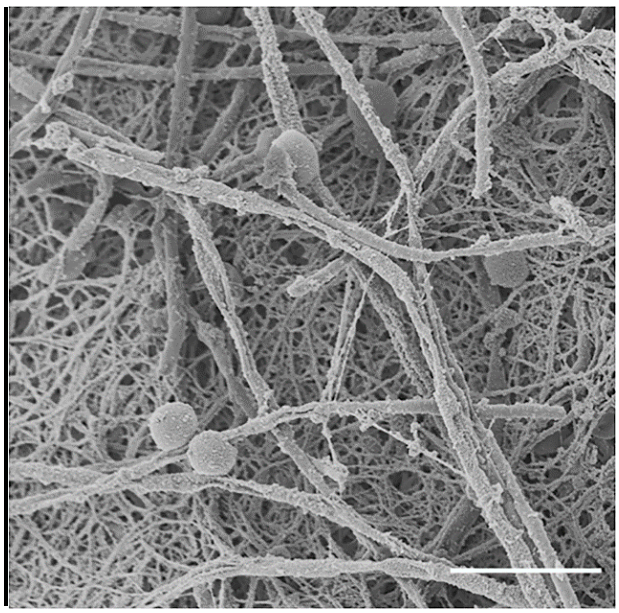

Figure 2: Fungal biofilms: Diagramed showing the organization of the fungal biofilm (PLOS).

Although there are few reports on biofilms, there are numerous studies that characterize the development of [2] in a variety of environments, including in niches in the environmental, industrial, and medical fields. structural features such as complex aggregated growth, surface-associated growth of cells, and secreted extracellular polymeric matrix and altered gene expression resulting in phenotypic changes that include enhanced tolerance to antimicrobial compounds or biocides, changes in enzyme or metabolite production, and/or secretion and physiological changes. The most frequent fungi to colonize implanted medical equipment are various Candida species.. On implanted medical equipment, Malessezia pachydermatis and Fusarium species also produce drug-resistant biofilms. The features of several fungi that are frequently linked to device-related biofilm infections [3].

Algal biofilms are extremely important to the environment, business, agriculture, and health, both from a helpful and a bothersome standpoint. Early studies on these biofilms tended to concentrate on how they grew out of control in water or on artificial structures. Researchers have concentrated their efforts on examining the importance of the multiscale interactions in environmental biology and agriculture as a result of improvements in scientific methods that have improved our understanding of these interactions. Algal biofilms serve as excellent models for understanding the interactions of various prokaryotic and eukaryotic partners as well as how they interact in soil and with plant tissues (Figure.3). This is especially important given their potential to increase crop output, fight disease, and enhance soil functionality. They serve as model systems for bioremediation and the production of useful products from harsh environments due to their ability to survive and operate even in these conditions. This compilation makes an effort to compile data on these intriguing biological entities and

emphasizes the need for in-depth examinations of their structure and function in order to effectively utilize them across a range of applications.

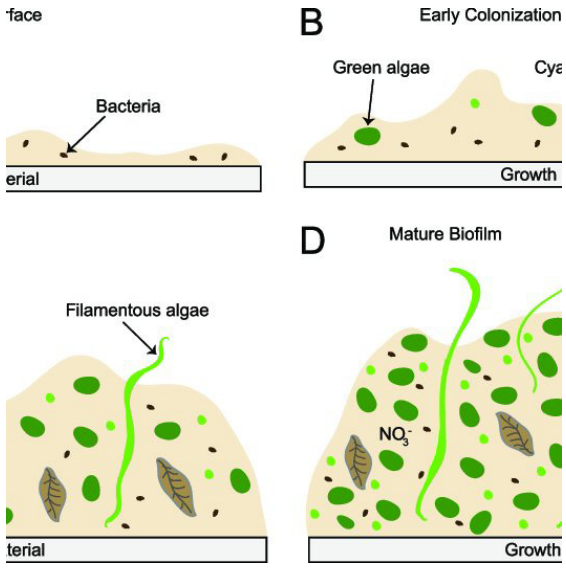

Figure 3: Algal biofilms: Diagramed showing the organization of the algal biofilm along with the bacterial biofilms (Research gate).

The biofilm offers the microorganisms a hospitable habitat. The cells remain in an ideal niche because of their adhesion to a surface with an increased nutrient supply. The cells' proximity makes it simple for them to communicate with one another via signal molecules. The possibility of horizontal gene transfer, or the exchange of genetic material between cells, is also enhanced by proximity. In conclusion, we talked about the various kinds of biofilms that are prevalent, as well as their nature and purposes.

LITERATURE REVIEW

Bacteria have the capacity to create biofilms as a universal trait. Multicellular colonies known as biofilms are held together by a self-made extracellular matrix. Different bacteria use different mechanisms to create biofilms, and these mechanisms frequently rely on the environment and particular strain characteristics. To provide an overview of how various organisms create biofilms, we focus on four extensively researched model systems in this review: *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis*, and *Staphylococcus aureus*. We address the essential characteristics of biofilms and the mechanisms by which extracellular signals cause biofilm formation using these bacteria as examples [4].

The complex communities known as surface-associated biofilms, are frequently built by and contain microbes. The type of the biofilm's resident microbes and the surrounding environment have an impact on its exact structure, chemistry, and physiology. However, a crucial similarity among biofilms is that an extracellular matrix made by their component cells plays a crucial role in maintaining their structural integrity. Extracellular matrices may be as diverse as biofilms and play a major role in the community's organization. Recent developments in our knowledge of the extracellular matrix and its function in biofilm biology are covered in this review [5].

Every aqueous system that supports life has been characterized as having biofilms, or collections of microorganisms at interfaces. These microbial communities can be monolayers of dispersed single cells or dense, macroscopic structures made of mucus (microbial mats; algal-microbial associations; trickling filter biofilms). In recent years, a wide range of

microscopic, physicochemical, and molecular biological techniques have been used to study and assess the structure of biofilms from many different environments, showing a typically complex 3D structure. Parallel to these studies, increasingly intricate mathematical simulations and models were created to describe the formation, organization, and interactions of biofilms. There is ongoing debate over the factors that affect how channels, microcolonies, and extracellular polymeric substances (EPS) shape the geographic structure of biofilms. Both modeling and experimental study must work together to come up with definitive explanations for the structures seen in biofilms. As molecular techniques advance, it is now possible to record single cells' functional activities in their biofilm environment as well as the spatial distribution of species in ever-greater detail. The mechanisms underlying the developmental processes involved in the formation and behavior of biofilms will undoubtedly be better understood using these novel techniques [6].

Our classical perception of microorganisms as unicellular life forms is almost entirely based on the pure-culture mode of growth; since microbial suspensions can be diluted to a single cell and studied in liquid culture, this mode of growth has traditionally predominated in the study of microbial physiology and pathogenesis in the research laboratory. However, many microbes are found in biofilm environments attached to surfaces rather than as free-floating (planktonic) creatures in their natural habitats. Consequently, structured microbial populations that are adhered to a surface and enclosed in an exopolymeric matrix are described as biofilms. This is especially important because it is currently thought that the formation of biofilms plays a substantial role in the majority of human microbial infections [7].

Candida albicans's capacity to create biofilms tightly knit colonies of cells adhered to a surface—is a key component of its virulence. Biofilm-associated infections are a major clinical issue because these biofilms are inherently resistant to conventional antifungal therapeutics, the host immune system, and other environmental factors. The formation, control, and molecular mechanisms of *C. albicans* biofilms are reviewed here [8].

A variety of microorganisms, including pathogens, create biofilms that give these organisms a way to defend themselves from antimicrobial agents. Several mechanisms have been proposed to explain this phenomenon of resistance within biofilms, including delayed penetration of the antimicrobial into the biofilm extracellular matrix, slowing of the growth rate of organisms within the biofilm, or other physiologic changes brought about by the interaction of the organisms with a surface. The practical ramifications of biofilm formation require the development of alternative control methods for determining the organisms' susceptibility to treatment as well as for treating an existing biofilm to change its structure. Numerous diagnostic procedures have been created. Effective treatment plans will include antimicrobials or other substances that have been shown to penetrate biofilms and destroy organisms within them, as well as therapies that interfere with or specifically target the biofilm matrix. More research is necessary to gain a clearer understanding of the function of biofilms in infection and how they react to particular treatments in vivo [9].

Understanding bacterial biofilms and their connection to human disease has received increasing interest. In the setting of the Gram-positive cocci, Staphylococcus aureus, we examine the genetic control and molecular elements involved in biofilm formation and maturation in this review. Along with illnesses and host immune reactions, we also go over current treatments for S. aureus biofilm infections as well as preventative measures. The development of *Staphylococcus aureus* biofilms is closely controlled by intricate genetic factors. The majority of host immune reactions to persistent biofilm infections are ineffectual and result in chronic illness. However, current research has considered biofilm development

to better understand host immunity to infection, and this may help create effective anti-biofilm S. aureus therapies [10].

An opportunistic human pathogen called *Pseudomonas aeruginosa* can cause severe acute and persistent infections in people with weakened immune systems. Its ability to create antibiotic-resistant biofilms is what is responsible for its extremely well-known persistence in clinical contexts. Biofilm is an architecture built mostly by autogenic extracellular polymeric substances which function as a scaffold to encase the bacteria together on surfaces, and to protect them from environmental stresses, impeding phagocytosis and thereby conferring the capacity for colonization and long-term persistence. Here, we summarize the current state of knowledge regarding *P. aeruginosa* biofilms, their stages of development, and the molecular mechanisms of invasion and persistence they bestow. Interspecies biofilms of *P. aeruginosa* and commensal Streptococcus that impede *P. aeruginosa* virulence and possibly improve disease conditions will also be addressed. Explosive cell lysis within bacterial biofilms to create essential communal materials. We'll look into recent studies on *P. aeruginosa* infection diagnosis. The final step will be to collect therapeutic approaches for the management of *P. aeruginosa* biofilms, along with their benefits and drawbacks [11].

Quorum sensing (QS), in which bacterial cells interact with one another by releasing, sensing, and reacting to small diffusible signal molecules, is known to control the cooperative behaviors and physiological processes of many bacteria. The ability of bacteria to interact socially and converse with one another like a multicellular organism has greatly aided bacterial host colonization, biofilm formation, competitive defense, and environmental adaptation.

A significant number of QS-controlled processes have been connected to the virulence and pathogenicity of microbes. Therefore, a novel method of preventing bacterial infections may be discovered by comprehending the molecular specifics of quorum sensing mechanisms and their regulated social activities [12].

CONCLUSION

In this chapter, we have discussed the species variety and the spatial distribution of various species in biomes. Understanding gene expression patterns, underlying physiological principles, and cell-to-cell interactions in biological systems will be a struggle in the future. A novel field for studying in depth the physiological activity and status of individual cells in a spatial order has emerged due to the rapid development of molecular tools. To comprehend the physiological and morphological potential of a "species," the regulatory processes (such as sensing and signaling) will therefore be further investigated. These studies will not only shed light on the complexity of biological communities, but they will also aid in the understanding of fundamental issues like "What does the viable but-non-culturable state" in the life cycle of bacteria mean regarding the proximity of biological communities, considerations regarding the potential for bacteria to experience complex morphological differentiation, always accompanied by physiological adaptations, are necessary. Finally, we study the discussed the different types so bacteria, algae, and fungi involved in the formation of biofilms in the environmental niche as well as the artificial system also.

REFERENCES:

[1] R. M. Donlan and J. W. Costerton, “Biofilms: Survival mechanisms of clinically relevant microorganisms,” *Clinical Microbiology Reviews*. 2002. doi: 10.1128/CMR.15.2.167-193.2002.

[2] T. B. Afonso, L. C. Simões, and N. Lima, "Occurrence of filamentous fungi in drinking water: their role on fungal-bacterial biofilm formation," *Research in Microbiology*. 2021. doi: 10.1016/j.resmic.2020.11.002.

[3] J. V. Desai, A. P. Mitchell, and D. R. Andes, "Fungal biofilms, drug resistance, and recurrent infection," *Cold Spring Harb. Perspect. Med.*, 2014, doi: 10.1101/cshperspect.a019729.

[4] S. Eick, "Biofilms," *Monographs in Oral Science*. 2020. doi: 10.1159/000510184.

[5] S. S. Branda, Å. Vik, L. Friedman, and R. Kolter, "Biofilms: The matrix revisited," *Trends in Microbiology*. 2005. doi: 10.1016/j.tim.2004.11.006.

[6] J. Wimpenny, W. Manz, and U. Szewzyk, "Heterogeneity in biofilms," *FEMS Microbiology Reviews*. 2000. doi: 10.1016/S0168-6445(00)00052-8.

[7] G. Ramage, S. P. Saville, D. P. Thomas, and J. L. López-Ribot, "Candida biofilms: An update," *Eukaryotic Cell*. 2005. doi: 10.1128/EC.4.4.633-638.2005.

[8] M. Gulati and C. J. Nobile, "Candida albicans biofilms: development, regulation, and molecular mechanisms," *Microbes and Infection*. 2016. doi: 10.1016/j.micinf.2016.01.002.

[9] R. M. Donlan, "Role of biofilms antimicrobial resistance," *ASAIO Journal*. 2000. doi: 10.1097/00002480-200011000-00037.

[10] J. M. Higashi and P. M. Sullam, "Staphylococcus aureus biofilms," in *Biofilms, Infection, and Antimicrobial Therapy*, 2005. doi: 10.4161/viru.2.5.17724.

[11] M. T. T. Thi, D. Wibowo, and B. H. A. Rehm, "Pseudomonas aeruginosa biofilms," *International Journal of Molecular Sciences*. 2020. doi: 10.3390/ijms21228671.

[12] Y. H. Li and X. Tian, "Quorum sensing and bacterial social interactions in biofilms," *Sensors*. 2012. doi: 10.3390/s120302519.

CHAPTER 15

OVERVIEW OF UNDERSTANDING HOW BIOFILMS EVADE HOST DEFENSE SYSTEM

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ABSTRACT:

Biofilms are communities or clusters of microbes that adhere to the surfaces of inanimate objects like catheters, prosthetic implants, or artificial heart valves as well as to animate objects like bones, tissues, and heart valves. Microbe communities found in biofilms vary significantly from those found in planktonic environments. Local antigen-presenting cells (APCs) launch the host defense program after a bacterial pathogen causes gut infection by phagocytosing invaders and causing localized swelling by secreting cytokines and chemokines. First, even though they are very susceptible to antibiotics as individual cells, microbes that exist in communities become much less susceptible to them. As a result, when microorganisms group, they are defended from a range of medicines that frequently recommend for treatment. Second, and more importantly from this viewpoint, these communities of microorganisms are resistant to the host immune system's assault and eradication.

KEYWORDS:

Aureus biofilms, Bacterial biofilm, Host cells, Immune response, Staphylococcal biofilms,

INTRODUCTION

The general idea of the unicellular lifestyle has historically been supported by the cultivation of microorganisms as free-floating, or "planktonic," cells in pure liquid cultures. A highly hydrated polysaccharide matrix, however, wasfound to be embedded in bacterial groupings in the late 1970s, mediating the bacteria's adhesion to solid aquatic surfaces. Several years later, the same research team called these cellular communities “biofilms,” defined as a functionally heterogeneous aggregate of microcolonies or single cells encased in a matrix of self-produced extracellular polymeric molecules that could adhere either to organic, abiotic surfaces or to each other. Microbial biofilms can grow into highly organized structures with pathways for the transportation of water, nutrients, and metabolic waste. Numerous genes are expressed as a result of adhesion to substrates or surfaces, and various cell aggregates within a biofilm have distinct gene expression patterns that control the growth and maturation of the biofilm. The idea that most, if not all, bacteria and fungi can form biofilms as a survival strategy in harsh environments has been the subject of extensive study since the 1980s. Biofilms offer protection from biotic and abiotic stresses. Surfaces exposed to or holding moisture and some nutrients are prime candidates for cell attachment and biofilm growth. River stones, oil and gas installations, ship hulls, water pipelines, food-processing surfaces, contaminated surgical tools, indwelling medical devices, human teeth, and infected wounds are examples of natural or artificial substrates for cell attachment and biofilm development [1].

According to models, the creation of mature biofilms goes through three stages: attachment, proliferation, and detachment or dispersal. Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which are staphylococcal surface-attached

proteins, engage non-covalently with host tissues and device surfaces during attachment. After attachment, proliferation, and maturation of the biofilm follow, with the production of an extracellular matrix consisting of the staphylococcal biofilm exopolysaccharide, polysaccharide intercellular adhesin (PIA), also called poly-N-acetylglucosamine (PNAG), teichoic acids, proteins, and extracellular DNA (eDNA). To facilitate nutrient delivery to the biofilm's deeper layers, channels, and mushroom-shaped structures develop during this second stage of biofilm expansion. The detachment and subsequent dispersal/dissemination of biofilm clusters to distal sites define the final stage of biofilm development, a process largely attributed to the activity of the surfactant-like phenol-soluble module (PSM) peptides (Figure .1).

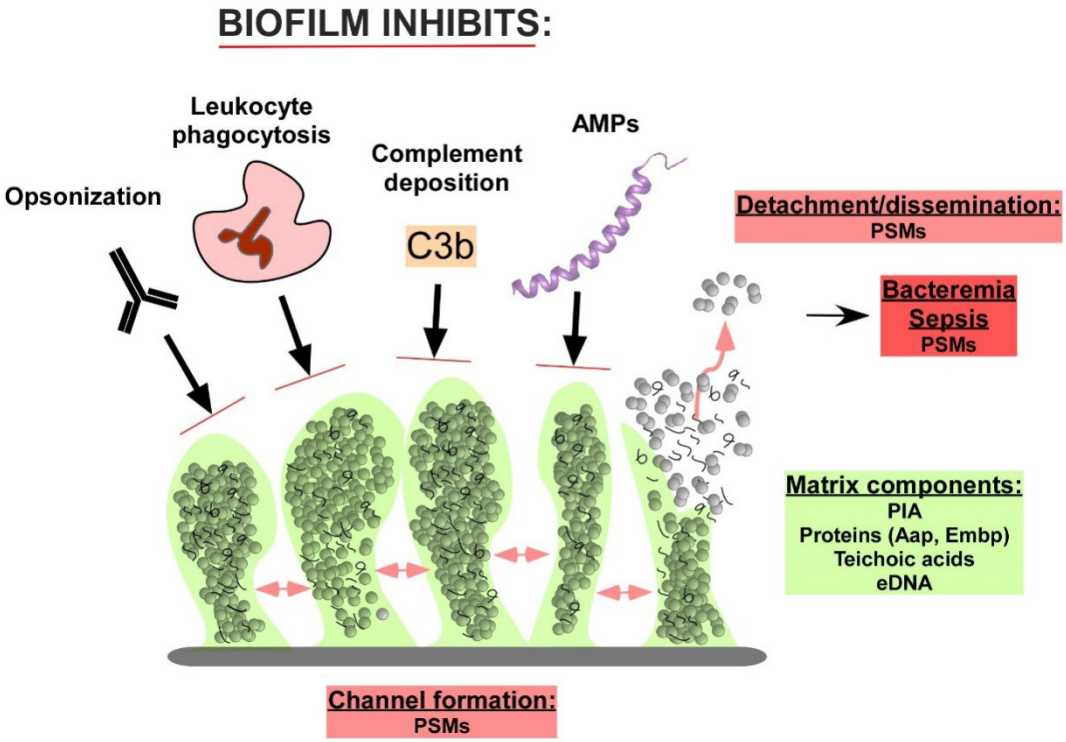

Figure.1: Bacteria and host cellular component interaction: Diagramed showing the key component involved in the bacteria host invasion (Frontier).

P. aeruginosa biofilms can also develop when this pathogen infects the cornea, burn victims' epidermis, implanted medical devices, and people who are also HIV-positive. The host immune system is mildly or seriously weakened in each of these situations. The innate immune system's primary antibacterial strategy relies on phagocytes, such as neutrophils and macrophages, engulfing and eliminating microbes. When pathogens exist as single, planktonic organisms, this defense mechanism is very successful against a wide variety of pathogens. However, thwarted phagocytosis, which occurs when phagocytes come into contact with bacteria in biofilms, makes this process less effective (Figure. 2). Such "frustrated" macrophages and neutrophils become activated and secrete toxic substances that harm adjacent healthy host tissues when they come into contact with but are unable to engulf bacteria in biofilms. In some instances, biofilms alter how well those effector molecules work. Less superoxide is produced by neutrophils when they come into contact with *P. aeruginosa* biofilms compared to when they come into contact with the pathogen's planktonic state. In reaction to *P. aeruginosa* biofilms, other oxygen-dependent (nitric oxide) and oxygen-independent host-neutrophil responses (lysozyme, lactoferrin) are also diminished in magnitude. Biofilm organisms generally modify the host response so that it is either

diminished in magnitude or ineffective against the bacterial community [2] even though the mechanisms underlying these reductions are not completely known.

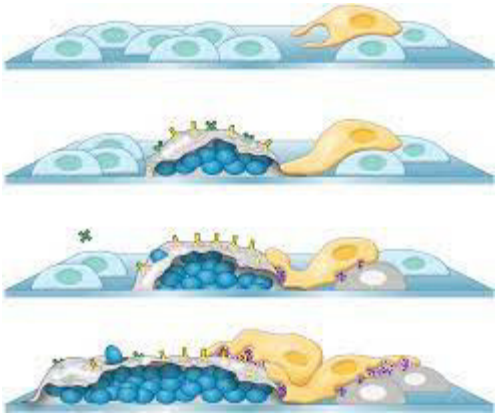

Figure 2: Defense mechanism:Graphic representation of blocked phagocytosis that takes place in response to biofilm microorganisms by phagocytes (Antimicrobe)

In the majority of environments, including those associated with human disease, natural biofilms coexist and create polymicrobial communities. Both synergistic and antagonistic interactions between bacterial and/or fungal taxa have been observed. The "coaggregation symbiosis" between *C. albicans* and *S. aureus*, in which Candida hyphal penetration through epithelial layers serves as a pathway for staphylococci, is an illustration of a mutually advantageous interaction. Additionally, the observed hyphal-mediated increased pathogenicity of *S. aureus* may be ascribed to the differential regulation of virulence factors generated during polymicrobial growth in addition to physical interactions. In a single biofilm community, various microbial species may provide passive resistance, metabolic cooperation, quorum sensing systems, and genotypic variability that give an edge to combat unfavorable environmental circumstances. *A. fumigatus* and *P. aeruginosa*, which are both found in the cystic fibrosis (CF) lung microbiome, are said to cooperate antagonistically. Direct contact with a heat-stable soluble factor secreted by *P. aeruginosa* inhibits the formation of *A. fumigatus* biofilms, indicating that small diffusible molecules can hinder filamentous fungal growth in environments with a variety of microorganisms. Although research has recently focused on polymicrobial biofilms, there is still much to learn about microbial cohabitation and how microbes engage with the host to reduce the effects of diseases linked to polymicrobial biofilms [1]. The diversity and life cycle of biofilms, as well as detection techniques for the emergence of biofilms and host immune reactions to pathogens, will all be covered in this chapter. Then, we'll concentrate on recent theories regarding immune evasion strategies in bacterial and fungal biofilms.

LITERATURE SURVEY

A complex matrix is where adherent bacterial populations known as biofilms are housed. Less is known about host immunity to staphylococcal biofilms and how they affect anti-bacterial effector mechanisms when arranged in this protective environment, even though host immune responses to planktonic staphylococcal species have been reasonably well characterized. Previously, it was believed that staphylococcal biofilms could avoid immune detection due to their persistent and passive character. Instead, we argue that staphylococcal biofilms skew the host immune response away from a bactericidal, pro-inflammatory phenotype and toward a pro-fibrotic, anti-inflammatory response that promotes bacterial persistence. Recent research from our group using a mouse model of catheter-associated biofilm infection supports this theory. *S. aureus* biofilms caused an accumulation of

alternatively activated M2 macrophages, which are pro-fibrotic and anti-inflammatory. Additionally, only a small number of neutrophils were drawn into *S. aureus* biofilms, illustrating yet another process distinct from planktonic infections. Though studies by others have shown the induction of various immune responses during staphylococcal biofilm growth in other models, indicating influences from the local tissue microenvironment, it is crucial to recognize the diversity of biofilm infections. The immune defenses that staphylococcal biofilms avoid as well as unresolved theoretical problems will be covered in this review. Targeted therapies to correct these flaws and hasten biofilm clearance may be developed as a result of a better grasp of why the host immune system is unable to eradicate biofilm infections [3].

The stages of the biofilm development cycle include the attachment of the microorganisms to the substrate, followed by a more lasting adhesion, the arrangement of microcolonies, and cell detachment necessary for the spread of single or clustered cells to other organ systems. For the detection and quantitation of biofilms, numerous techniques have been devised. In tissue culture plates, silicone tubes, staining techniques, and direct inspection with scanning electron microscopy or confocal scanning laser microscopy are all ways to find microbes that produce biofilms. Methods used to quantify biofilm development include DNA quantification, colony-forming unit counting, dry cell weight assays, and the XTT 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide reduction assay. Through effector mechanisms mediated by immune cells, receptors, and several humoral factors, innate immune defense strategies are able to create an immediate response in the event of infection. We give an overview of the diversity and life cycle of biofilms, as well as detection techniques for the emergence of biofilms and host immune reactions to pathogens. Then, we concentrate on recent theories regarding immune evasion strategies in bacterial and fungal biofilms. This seems to be especially important given that using host immune reactions as a form of treatment for biofilms may be novel [1].

Pseudomonas aeruginosa, an opportunistic gram-negative bacterium, is easily isolated from chronic wounds, medical equipment, and the lungs of cystic fibrosis patients. It is involved in several chronic infections. Due to P. aeruginosa's ability to create biofilms, which shield the agglomerated, biopolymer-embedded bacteria from the negative effects of antibiotic treatments and host immunity, it is thought that *P. aeruginosa* can survive in the host organism. Rhamnolipid, a virulence factor controlled by quorum sensing (QS), is a crucial element in the defense against natural immunity. QS is a cell-to-cell signaling system that synchronizes virulence expression with the defense of gathered biofilm cells. Rhamnolipids are recognized for their capacity to induce hemolysis and have been demonstrated to do so in a number of immune system cells, including macrophages and polymorphonuclear leukocytes. (PMNs). The interaction of *P. aeruginosa* and PMNs in chronic infections is addressed in this chapter with an emphasis on the function of rhamnolipids and extracellular DNA [4].

A significant contributor to nosocomial morbidity and death is Candida biofilms. It is still unclear how Candida biofilms manage to escape the immune system. To explain how biofilms can evade host immunity, we create a theoretical framework of three, not mutually exclusive, models. First, the immune response may be prevented by biofilms' immunological silence characteristics. Second, immune-deviating factors produced by biofilms may turn effective immunity into ineffectual immunity. Third, despite being otherwise successful, host immunity may not work against biofilms. We discovered that mice infected with biofilms acquired sterilizing immunity when challenged with yeast from Candida using a murine subcutaneous biofilm model. Even though an efficient anti-Candida immunity was induced,

no natural clearance of the biofilm was seen. These findings show an asymmetric connection between the host and biofilms, with biofilms evoking powerful immune responses while resisting immunological clearance [5], supporting the immune resistance model of biofilm immune evasion.

Extracellular opportunistic bacterium *Pseudomonas aeruginosa* employs two main strategies to get around the host defense system. Production of numerous extracellular products like lipases, toxins, and proteases is one of these methods. Alkaline protease and elastase, two proteases, suppress the activity of immune system cells (phagocytes, NK cells, and T cells), inactivate several cytokines (IL-1, IL-2, IFN-r, and TNF), cleave immunoglobulins, and render complement inactive. Bacterial proteases' inhibition of the local immune response creates an environment favorable for colonization and the development of chronic infection. The bacterial development in biofilms during chronic infections is another way that *P. aeruginosa* evades the host defense system. Low phagocyte responses are induced by bacteria growing in biofilms, which serve as a barrier for the bacteria against complement, antibodies, and immune system cells. The main causes of P. aeruginosa's persistence in chronic infections are protection from the host defense system and greater antibiotic tolerance of the bacteria in the biofilm [6].

Most hospital-acquired illnesses are caused by infections connected to medical devices. Depending on the type of implant and the anatomical location of implantation, a variety of opportunistic pathogens can result in implant infections. These adaptable pathogens must quickly cling to almost all biomaterial surfaces and endure in the adverse host environment in order to succeed. Implant surface biofilm formation protects the microbes and promotes infection persistence. Additionally, bacteria that cause implant infections are capable of evading both natural and adaptive host defenses as well as biocides and antibiotic chemotherapies. Orthopedic implants and Staphylococcus aureus serve as good example as we examine the basic pathogenic mechanisms underlying implant infections in this review. We also talk about creative targets for preventive and therapeutic approaches [7].

Complex bacterial communities known as biofilms are enclosed in a matrix mainly made of polysaccharides, extracellular DNA, and protein. Biofilm infections caused by Staphylococcus aureus can develop and are frequently incapacitating because of their chronic nature and resistance to antibiotic treatment. The immune responses triggered by biofilm development and how they affect bacterial elimination are still unknown. Because ligands for both TLR2 and TLR9 are found within the biofilm, we used a mouse model of catheter-associated biofilm infection to evaluate the functional significance of TLR2 and TLR9 in the host immune response during biofilm development. It's interesting to note that neither TLR2 nor TLR9 affected bacterial abundance or the release of inflammatory mediators during in vivo biofilm development, indicating that S. aureus biofilms avoid these conventional bacterial recognition pathways. Several potential mechanisms were identified to account for biofilm evasion of innate immunity, including significant reductions in IL-1β, TNF-α, CXCL2, and CCL2 expression during biofilm infection compared with the wound healing response elicited by sterile catheters, limited macrophage invasion into biofilms in vivo, and a skewing of the immune response away from a microbicidal phenotype as evidenced by decreases in inducible NO synthase expression concomitant with robust arginase-1 induction. Macrophages that were effective at invading S. aureus biofilms showed limited phagocytosis and gene expression patterns resembling those of alternatively activated M2 macrophages, according to in vitro coculture studies. These results show that S. aureus biofilms can reduce the pro-inflammatory responses of the host, which may help to explain why biofilm infections persist in immunocompetent hosts [8].

Microbial colonies known as biofilms develop on surfaces and are enmeshed in an extracellular matrix. When host immunity or mucosal ecology shift, *C. albicans* produces pathogenic mucosal biofilms. These biofilms are polymicrobial because numerous microbial species live on mucosal surfaces. Recent research has used biofilm analysis paradigms to investigate mucosal *C. albicans* infections. Even though the most important Bcr1 target genes can change depending on the biofilm niche, these studies demonstrate that the Bcr1 transcription factor is a master regulator of *C. albicans* biofilm formation under a variety of circumstances. The interaction with host defenses is a crucial factor in determining the development of mucosal biofilms. Finally, research on the interactions between different bacterial species and *C. albicans* sheds light on the communication pathways that give polymicrobial biofilms their distinctive characteristics [9].

CONCLUSION

For 80% of bacterial infections, a population of bacteria called a biofilm that is embedded in the extracellular matrix is responsible. When micronutrients and local oxygen are scarce, biofilm allows bacterial cells to create specific conditions and produce virulence determinants, which makes them resistant to different antibacterial agents. Additionally, the human defense system is not entirely effective in removing biofilm. Most importantly, a growing body of research demonstrates that some bacterial species use several mechanisms to commandeer the components of the host to create a biofilm. In this regard, host components, such as DNA, hyaluronan, collagen, fibronectin, mucin, oligosaccharide moieties, filamentous polymers (F-actin), plasma, platelets, keratin, sialic acid, laminin, vitronectin, C3- and C4- binding proteins, antibody, proteases, factor I, factor H, and acidic proline-rich proteins have been reviewed. Therefore, to successfully treat biofilm-associated infections, it would be essential to characterize how bacteria and their biofilm interact with the host. In this paper, we review the most recent data on how bacteria create biofilm by appropriating host factors.

REFERENCES:

[1] E. Roilides, M. Simitsopoulou, A. Katragkou, and T. J. Walsh, “How Biofilms Evade Host Defenses,” *Microbiol. Spectr.*, 2015, doi: 10.1128/microbiolspec.mb-0012-2014.

[2] J. G. Leid, “Bacterial biofilms resist key host defenses,” *Microbe*, 2009.

[3] M. L. Hanke and T. Kielian, “Deciphering mechanisms of staphylococcal biofilm evasion of host immunity.,” *Frontiers in cellular and infection microbiology*. 2012. doi: 10.3389/fcimb.2012.00062.

[4] M. Alhede, T. Bjarnsholt, M. Givskov, and M. Alhede, “Pseudomonas aeruginosa biofilms. Mechanisms of immune evasion,” in *Advances in Applied Microbiology*, 2014. doi: 10.1016/B978-0-12-800262-9.00001-9.

[5] J. E. Garcia-Perez *et al.*, “A framework for understanding the evasion of host immunity by Candida biofilms,” *Frontiers in Immunology*. 2018. doi: 10.3389/fimmu.2018.00538.

[6] A. Kharazmi, “Mechanisms involved in the evasion of the host defence by Pseudomonas aeruginosa,” *Immunol. Lett.*, 1991, doi: 10.1016/0165-2478(91)90026-7.

[7] C. R. Arciola, D. Campoccia, and L. Montanaro, “Implant infections: Adhesion, biofilm formation and immune evasion,” *Nature Reviews Microbiology*. 2018. doi: 10.1038/s41579-018-0019-y.

[8] L. R. Thurlow *et al.*, " Staphylococcus aureus Biofilms Prevent Macrophage Phagocytosis and Attenuate Inflammation In Vivo ," *J. Immunol.*, 2011, doi: 10.4049/jimmunol.1002794.

[9] S. Ganguly and A. P. Mitchell, "Mucosal biofilms of Candida albicans," *Current Opinion in Microbiology*. 2011. doi: 10.1016/j.mib.2011.06.001.

CHAPTER 16

CANDIDA BIOFILMS DEVELOPMENTS, INFECTION, AND RESISTANCE AGAINST THE DRUG

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ABSTRACT:

The main fungus that affects people, *Candida*, causes a wide range of illnesses, from superficial mucosal conditions to serious mycoses. *Candida's* ability to create biofilms is a key component of its pathogenicity, and these biofilms are particularly challenging to remove due to their extremely high antifungal resistance. As a result, studies into the pathogenicity of *Candida* have concentrated on biofilm management, antifungal resistance, and prevention of their formation. Although studies have provided some insight, a complete understanding of the molecular processes that control biofilm formation and pathogenicity is still awaited. The main aspects of what is presently understood about Candida biofilm development, regulation, antifungal resistance, and proteomics are outlined in this review.

KEYWORDS:

Antifungal Resistance, Albicans Biofilms,*Candida Albicans,* Fungal Biofilms, Fungal Infection.

INTRODUCTION

For the hospital community, fungi are a major source of infection. The most significant risk factors for invasive fungal infection include the use of broad-spectrum drugs, parenteral nutrition, indwelling catheters, immunosuppression, and disruption of mucosal barriers as a result of surgery, chemotherapy, and radiation. Candida bloodstream infection is the most frequent etiologic agent of fungal-related biofilm infection and the third most frequent cause of nosocomial bacteremia in patients needing critical care [1]. The most common cause of biofilm development is *C. albicans*, a common commensal of human mucosal surfaces and an opportunistic pathogen in immunocompromised patients. Acute fungemia and/or widespread infection can be caused by cells that have become detached from adherent biofilm structures that have developed on indwelling medical devices like intravascular catheters. Recent research has revealed that cells that separate from biofilms are more likely to die than comparable planktonic yeasts [2].

It may be necessary to remove the implant physically in order to treat these infections that are linked to implants because they are naturally difficult to treat. Other nonalbicans Candida species include *C. glabrata, C. parapsilosis, C. dubliniensis, C. krusei*, and *C.tropicalis*, which are linked to biofilm development and catheter-related bloodstream or device-related illnesses.Infections caused by yeasts and filamentous fungi, such as *Pneumocystis, Coccidioides, Aspergillus, Zygomycetes, Blastoschizomyces, Saccharomyces, Malassezia, Trichosporon*, *Cryptococcus*, have also been reported more frequently. It has been demonstrated that *Cryptococcus neoformans* can colonize and then create biofilms on cardiac valves, prosthetic hip joints, peritoneal dialysis fistulas, and ventricular shunts. As a result of biofilm-related illnesses, various Trichosporon species, including those that affect cardiac

grafts, catheters, and breast implants, can spread deadly infections (Figure.1). *Malassezia pachydermatis* has been isolated from patients undergoing parenteral nutrition, *Blastoschizomyces capitatus* has been associated with catheter-related fungemia , Saccharomyces cerevisiae has been detected from dentures of *stomatitis patients* , and recurrent meningitis has been associated with a *Coccidioides immitis* biofilm at the tip of a ventriculoperitoneal shunt tubing.

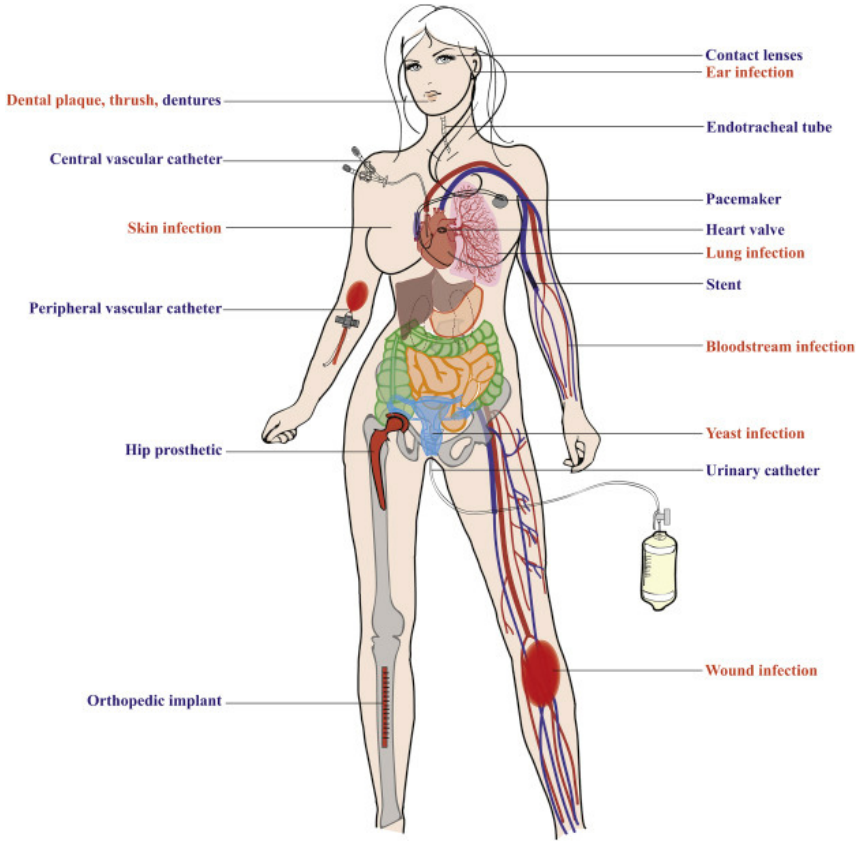

Figure 1: *C. albicans*-related infection. Diagramed showing the different sites of the infection related to the *C. albicans* (nature).

Additionally, there are an increasing number of cases linking biofilm infections to the filamentous mold *Aspergillus fumigatus*. For instance, it can result in an aspergilloma, a localized infection marked by a spherical mass of hyphae, in the respiratory system. There have also been reports of *aspergillary bronchitis*, which is indicated by bronchial deposits that contain mucus and mycelia. When examined histologically, bronchopulmonary lavage (BAL) of aspergillosis patients may also show the presence of numerous hyphae in the shape of a complex multicellular mycetoma structure sample (Figure.1). Additionally, it has been documented to result in severe biomaterial-related infections of breast augmentation implants, heart valves, catheters, cardiac pacemakers, and joint replacement implants. While less commonly linked to *A. fumigatus*, aspergillomas have been found to support the urinary tract. Complex nasal infections, which in dogs have been referred to as superficial mucosal fungal plaque, are also frequently linked to it.

Our understanding of fungal biofilms has significantly increased as it has become more obvious that a wide range of fungi can create them. We now have a better understanding of the molecular characteristics of fungal biofilm development thanks to work mainly with *C. albicans*. These have clinical significance because they are resistant to antifungal therapy, which presents a significant challenge to clinicians. After all, the dose necessary to eradicate

the biofilm can be greater than the maximum therapeutically achievable concentrations of antibiotics. The purpose of this paper is to present a current understanding of the major causes of antifungal agents' ineffectiveness against microbial biofilms[1].

The infamous resistance of microbial biofilms to a variety of antimicrobial agents, such as antibiotics, antiseptics, and industrial biocides, is arguably their most important characteristic. Bacteria that live as biofilms, for instance, are 10-1000 times more resistant to antibiotics than planktonic bacteria. It was first shown in 1995 that Candida biofilms exhibited corresponding resilience to antifungal agents. Amphotericin B, fluconazole, flucytosine, itraconazole, and ketoconazole were evaluated using a catheter disc assay along with other clinically significant antifungal medications. When compared to planktonic cells, all of these compounds were much less active against *C. albicans* biofilms. Drug resistance was also present in the biofilms of non-C. albicans species like C. tropicalis and C. parapsilosis. Drug resistance has been seen in later experiments when Candida biofilms are grown on surfaces like cellulose, polystyrene, and denture acrylic. However, it has recently been asserted that some of the more modern antifungal medications are effective against Candida biofilms. Although two new triazoles (voriconazole and ravuconazole) could not break down the biofilms of *Candida albicans* and *Candida parapsilosis*, they did appear to have some anti-biofilm action when combined with two echinocandins. (caspofungin and micafungin). If these intriguing results are confirmed, significant advancements in the treatment of fungal implant infections may result[2].

The study of monospecies biofilms, which have been characterized in both in vitro and in vivo systems and comprise four distinct phases of development, is where the majority of our understanding of *C. albicans* biofilm formation comes from. (Figure. 2). Round yeast cells stick to a solid surface to start the process of *C. albicans* biofilm development in the laboratory, a small silicone disc, the material of common intravascular catheters, or a polystyrene microtiter plate, are often used). Usually, a *C. albicans* culture is applied to the solid surface to start the adherence phase (60–90 minutes), and non-adhered or loosely adhered cells are then removed. This causes a basal layer of anchoring yeast cells to develop. (Figure. 2A).

Its life cycle is crucial for typical biofilm development and is frequently referred to as the "seeding" phase. Cell proliferation and early-stage adhesion cell filamentation make up the next step of biofilm development. (Figure. 2B). This is followed by biofilm maturation, resulting in a complex network of several layers of polymorphic cells, including hyphal cells (chains of cylindrical cells), pseudohyphal cells (ellipsoidal cells joined end to end), and round yeast cells, encased in an extracellular matrix, giving the biofilm a thick and structured appearance as well as providing protection from chemical and physical injury (Figure. 2C). In most cases, a mature biofilm takes 24 hours to develop.

It can be seen by the naked eye as a cloudy surface structure on top of the solid surface and under a microscope as a well-organized assemblage of various cell types. The growth media is continuously shaken or continuously pumped over the biofilm during these stages of biofilm development to imitate the flow conditions found in catheters and prevent free-floating cells from adhering to the surface. The dispersal stage, which occurs at the end of biofilm development and is known as the least understood stage of *C. albicans* biofilm development, is where some round yeast cells scatter from the biofilm to seed new locations (Figure. 2D). Studies have examined the effects of various substrate types, nutrient media, and the presence of flow or static conditions on biofilm development. Several models of in vitro *C. albicans* biofilm formation have been described. *C. albicans* biofilms can grow in the lab on a variety of substrates and in a variety of media, demonstrating the intrinsic

adaptability of biofilm development to a broad range of environmental conditions [3]. Here, we reviewed the state of information regarding *C. albicans* biofilm development, infection, and drug resistance.

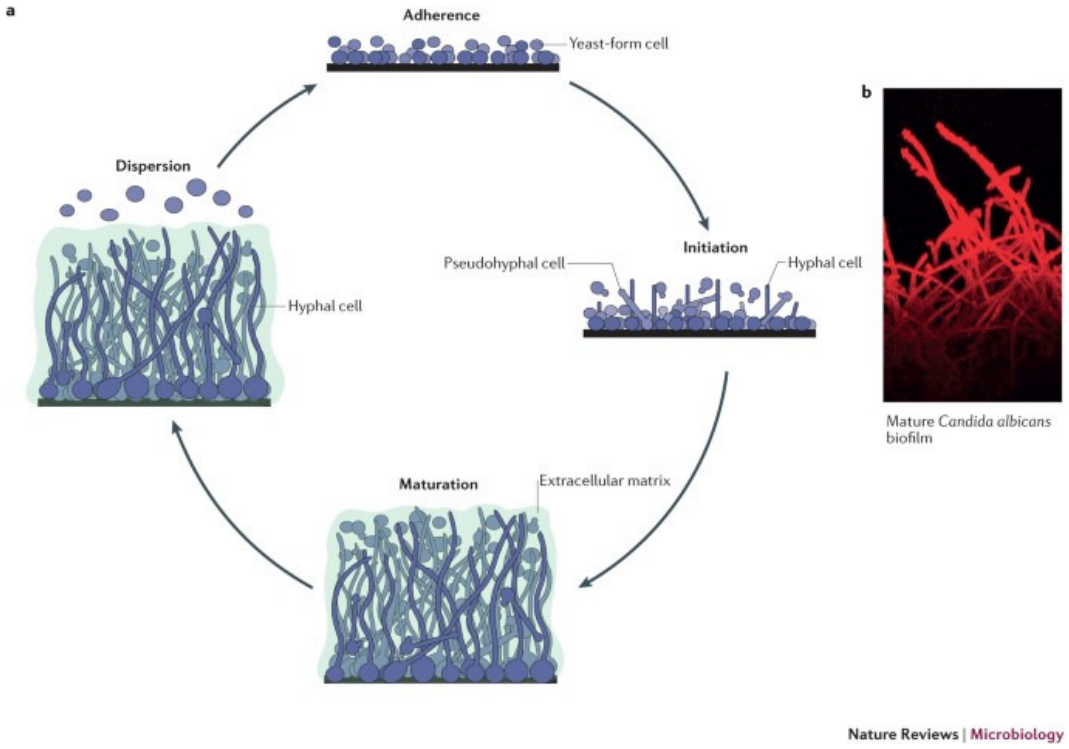

Figure: 2: *C. albicans*: Diagrame showing the life cycle of the *C. albicans* (Nature).

LITERATURE SURVEY

Fungal cells create biofilms, three-dimensional structures made up of cells encased in exopolymeric matrices, in reaction to the attachment to a surface. Candida albicans cells that adhere to surfaces go into a unique physiological state where they exhibit the drug efflux determinants CDR1, CDR2, and MDR1 and are extremely resistant to antifungal medications. The cellular morphology and matrix composition of *C. albicans* biofilms produced under various circumstances vary, which indicates that biofilms formed within a host, such as on indwelling medical devices, would also vary depending on the type of device and its location. Currently, it is unknown how surface attachment results in the development of biofilms [4].

Pathogenic fungi in the genus Candida are significant contributors to hospital-acquired infections and can result in both superficial and severe systemic diseases. Biofilms can develop on implanted devices like prosthetic heart valves or indwelling catheters during many Candida illnesses. Candida albicans biofilms are composed of yeast and hyphal microcolonies that are enclosed in a matrix and organized in a bilayer structure. Amphotericin B and fluconazole are just two of the antifungal medications that the biofilms are resistant to, and it appears that there are numerous pathways of resistance as well. Recent research with mixed biofilms of Candida and bacterial species suggests that prokaryotic and eukaryotic cells interact extensively and strikingly in these attached populations [5].

On implanted devices like a denture, a prosthetic heart valve, or an indwelling catheter, biofilm can develop during many Candida infections. Several model systems can be used to produce Candida biofilms in vitro. The simplest of these involves the growth of organisms on the surfaces of tiny spheres made of denture acrylic or catheter material. This method

produces C. albicans biofilms that are composed of bilayer-shaped, matrix-enclosed microcolonies that contain yeast hyphae and pseudohyphae. Several antifungal medications currently used in therapeutic settings, such as fluconazole and amphotericin B, are not effective against Candida biofilms. Multiple processes may be at play in biofilm drug resistance, according to recent research [2].

On catheters and other prosthetic devices, fungi of the genus Candida create biofilms. Due to their inherent resilience to almost all clinically used antifungals, these three-dimensional structures made up of yeast and hyphal cells embedded in an extracellular matrix represent a significant hurdle in the treatment of disseminated Candida infections. Candida biofilms are particularly robust to azoles and amphotericin B, but they are still vulnerable to the recently developed echinocandins that target the synthesis of cell wall -glucan. Biofilms' antifungal resistance is most likely the result of the interaction of several mechanisms that function in a time-dependent way. While changes in the sterol composition of membranes may account for the resistance of mature biofilms, drug efflux is expected to add to resistance during the early stages of biofilm formation. Gene expression patterns that reflect the initial physiology of mature Candida biofilms may help identify the genes necessary for the development of pleiotropic antifungal resistance [6].

The increased resistance of microbial biofilms to antimicrobial chemotherapies is one of their primary characteristics. The phenotypic changes that take place as a result of the switch from the planktonic to the biofilm mode of development are, however, still largely unknown at this time. *Candida albicans* biofilms had a well-organized three-dimensional structure and were made up of a thick network of filamentous cells and yeasts that were firmly enmeshed in an exopolymeric matrix. Fluconazole was inherently unsuitable for these biofilms. Additionally, when sessile cells were resuspended as free-floating cells, the resistance phenotype was still present, proving that exopolymeric material and biofilm stability are not the only factors affecting biofilm resistance. One of the primary methods of azole resistance in *C. albicans* under planktonic conditions is through active efflux of these medications, which is mediated by ATP-binding cassette (ABC) transporters and major facilitators. The ABC transporters and major facilitators, which are encoded by the CDR and MDR genes respectively, are two distinct kinds of efflux pumps.

In this research, we used northern hybridization to track the expression of these genes in populations of Candida albicans under both planktonic and biofilm growth. It was shown that during the process of biofilm formation and growth, the expression of genes encoding both varieties of efflux pumps was up-regulated. Additionally, to ascertain their role in biofilm resilience, the antifungal susceptibilities of biofilms produced by a collection of *C. albicans* mutant strains lacking efflux pumps were examined. Surprisingly, mutants bearing single and double deletion mutations in the genes cdr1, cdr2, mdr1, cdr1/cdr2, and mdr1/cdr1 were hypersensitive to fluconazole when planktonic but continued to exhibit the resistant phenotype during biofilm growth. These analyses show that *C. albicans* biofilm resistance is a multifaceted phenomenon that cannot be fully explained by a single mechanism alone. It may also entail different molecular mechanisms of resistance than those exhibited by planktonic cells [7].

The structure of *Candida albicans* biofilms was examined using two model biofilm systems that involved the development of either cylindrical cellulose filters or disks of catheter material. Two wild-type strains and two morphological mutants, which are deficient in yeast and hyphal growth, respectively, were used to create biofilms, which were then compared to gauge the significance of dimorphism in biofilm formation. A thin, basal yeast layer and a thicker, but more open, hyphal layer made up the biofilms of the wild-type strains that

formed on catheter disks, according to scanning electron microscopy and thin sections of biofilms studied by light microscopy. The yeast-mutant created a thicker, hyphal biofilm that was equivalent to the outer zone of the wild-type structures, whereas the hypha-mutant only produced the basal layer. The basal yeast layer may play a key role in securing the biofilm to the surface because the yeast-mutant biofilms were easier to separate from the catheter surface than the others. The appearance of the biofilms that developed on the cylindrical cellulose filters was very distinct. The yeast-mutant created a dense hyphal mat on top of the filter, whereas the hypha-mutant and both wild types only formed yeast-form biofilms. Regardless of their morphological makeup, all of these biofilms were immune to the antifungal medication amphotericin B. Overall, these findings suggest that a *C. albicans* biofilm's structure depends on the nature of the contact surface, but that some surfaces generate biofilms with layered architectures that are similar to those described for bacterial systems [8].

Single-species biofilms have been thoroughly studied in the majority of reductionist investigations of biofilm biology. However, biofilms in nature are typically composed of multiple species, where interspecies interactions can affect how these communities form, are structured, and work in contrast to biofilm populations. In order to investigate how interspecies interactions influence biofilm development, structure, and stress responses, a reproducible mixed-species biofilm made up of *Pseudomonas aeruginosa*, *Pseudomonas protegens*, and *Klebsiella pneumoniae* was modified. To identify each species' abundance and geographic localization within the biofilm, each was fluorescently tagged. Different structures in the mixed-species biofilm stood out from those in similar single-species biofilms. Additionally, compared to single-species biofilms, the formation of the mixed-species biofilm took 1-2 days longer.

Along the flow cell canal, where nutrient conditions and each species' growth rate may have an impact on community assembly, the composition and spatial structure of the mixed-species biofilm also changed. Strangely, compared to single-species biofilms, the mixed-species biofilms were more immune to the antimicrobials sodium dodecyl sulfate and tobramycin. Importantly, it was discovered that such community resilience was not the result of selection for the resistant species but rather a security provided to the entire community by the resistant species. In comparison, mixed-species planktonic cultures did not exhibit community-level resilience. These results imply that the structured biofilm community, where members are tightly entwined, is the only one that engages in community-level interactions, such as sharing of common resources [9].

Infections caused by fungus biofilms are now widely acknowledged to be serious health issues. One of the main causes of this is how they affect medical care because antifungal therapy frequently fails and requires surgical involvement. The cost of providing healthcare is significantly increased as a result. This paper aims to illustrate the importance of fungal biofilms, particularly *Candida albicans*, and discusses some of the key fungal biofilm resistance mechanisms that include, extracellular matrix (ECM), efflux pump activity, persisters, cell density, and overexpression of drug targets, stress responses, and the general physiology of the cell. The paper highlights the complexity of fungal biofilm resilience, which includes some of the most recent findings and theories in the area [1].

According to [3H]leucine incorporation and tetrazolium reduction assays, Candida albicans biofilms grown on tiny discs of catheter material were resistant to the effects of five therapeutically significant antifungal medications. The most effective drug against biofilm bacteria was fluconazole, and the least effective drug was amphotericin B. The biofilms' scanning electron microscopy supported these conclusions [10].

CONCLUSION

Microorganisms can hide in biofilms where they are secure from antibiotics and can act as a source of persistent infection. With the help of two clinically useful models of *Candida albicans* biofilms grown on bioprosthetic materials, we were able to show that biofilm formation involves three different stages. Adherent blastospores become well-defined cellular communities encased in a polysaccharide matrix during these growth stages. *C. albicans* biofilms were discovered to have a highly heterogeneous architecture made up of cellular and noncellular components using fluorescence and confocal scanning laser imaging. Antifungal resistance of biofilm-grown cells grew concurrently with biofilm formation in both models. Planktonic and biofilm-grown cells had different expression patterns for the agglutinin-like (ALS) genes, which produce a family of proteins involved in adhesion to host surfaces. Saccharomyces cerevisiae, which adhered to bioprosthetic surfaces but was unable to develop a mature biofilm, contrasts sharply with C. albicans' capacity to create biofilms. The studies discussed here serve as a foundation for research into the molecular processes underlying Candida biofilm biology and antifungal resistance. They also give us the tools we need to develop new treatments for infections caused by biofilms.

REFERENCES:

[1] G. Ramage, R. Rajendran, L. Sherry, and C. Williams, "Fungal biofilm resistance," *International Journal of Microbiology*. 2012. doi: 10.1155/2012/528521.

[2] L. J. Douglas, "Medical importance of biofilms in Candida infections," *Revista Iberoamericana de Micologia*. 2002.

[3] M. Gulati and C. J. Nobile, "Candida albicans biofilms: development, regulation, and molecular mechanisms," *Microbes and Infection*. 2016. doi: 10.1016/j.micinf.2016.01.002.

[4] C. A. Kumamoto, "Candida biofilms," *Current Opinion in Microbiology*. 2002. doi: 10.1016/S1369-5274(02)00371-5.

[5] L. J. Douglas, "Candida biofilms and their role in infection," *Trends in Microbiology*. 2003. doi: 10.1016/S0966-842X(02)00002-1.

[6] C. d'Enfert, "Biofilms and their Role in the Resistance of Pathogenic Candida to Antifungal Agents," *Curr. Drug Targets*, 2006, doi: 10.2174/138945006776359458.

[7] G. Ramage, S. Bachmann, T. F. Patterson, B. L. Wickes, and J. L. López-Ribot, "Investigation of multidrug efflux pumps in relation to fluconazole resistance in Candida albicans biofilms," *J. Antimicrob. Chemother.*, 2002, doi: 10.1093/jac/dkf049.

[8] G. S. Baillie and L. J. Douglas, "Role of dimorphism in the development of Candida albicans biofilms," *J. Med. Microbiol.*, 1999, doi: 10.1099/00222615-48-7-671.

[9] K. W. K. Lee, S. Periasamy, M. Mukherjee, C. Xie, S. Kjelleberg, and S. A. Rice, "Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm," *ISME J.*, 2014, doi: 10.1038/ismej.2013.194.

[10] S. P. Hawser and L. J. Douglas, "Resistance of Candida albicans biofilms to antifungal agents in vitro," *Antimicrob. Agents Chemother.*, 1995, doi: 10.1128/AAC.39.9.2128.

CHAPTER 17

ROLE OF THE BIOFILMS IN THE INFECTIOUS DISEASE

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ABSTRACT:

This chapter discussed the mutualistic, commensal, and parasitic functions that biofilms play in human biology and health. There is a growing understanding that several chronic inflammatory diseases that are not caused by devices are also linked to biofilm. Cystic fibrosis, chronic obstructive pulmonary disease (COPD), otitis media, and prostatitis are just a few of the diseases that are commonly known and evident. The typical method for identifying antibiotic sensitivities is to look at the area around a disc holding the target antibiotic where planktonic growth is inhibited. This chapter deals primarily with topics: how the phenotypic and genotypic characterization of biofilm bacteria has provided the data for the development of a new conceptual framework for the understanding of chronic infections and the medical effects of biofilm formation on host tissues and implanted medical devices. Bacterial biofilms' refractoriness to almost all host defense mechanisms and conventional treatments, including antibiotics, is their defining clinical characteristic. Following the removal of a single prosthetic joint due to biofilm infection, hundreds of thousands of dollars' worth of drugs are given intravenously. When the usual vaginal flora is disrupted, pathogenic bacteria and fungi overgrow, causing bacterial and candidal vaginosis.

KEYWORDS:

Bacterial Biofilms, Bacterial Infection, Chronic Infection, Infectious Disease, Medical Device.

INTRODUCTION

A biofilm is a collection of microbial cells that are encased in a polysaccharide-based matrix and are permanently attached to a surface (i.e., cannot be removed by mild rinsing). Depending on the context in which the biofilm has formed, noncellular substances such as mineral crystals, corrosion ppapers, clay or silt ppapers, or blood components may also be present in the biofilm matrix. The genes are transcribed differently in biofilm-associated organisms compared to their planktonic (freely suspended) peers. Biofilms can develop on a variety of surfaces, including living tissues, pipelines in industrial or potable water systems, and aquatic ecosystems in the wild. Scanning electron micrographs of biofilms from a medical device and an industrial water system, respectively, show the variable character of biofilms. The biofilm in the water system is extremely complicated and contains filamentous bacteria, freshwater diatoms, clay material, and corrosion products. Contrarily, the biofilm on the medical device seems to be made up of a singular, coccoid organism and the extracellular polymeric substance (EPS) matrix that is connected to it [1].

Bacterial biofilms are thought to be involved in about 65% of all bacterial illnesses (Figure.1). Both device- and non-device-associated illnesses fall under this category. For several devices, such as 2% for breast implants, 2% for joint prostheses, 4% for mechanical heart valves, 10% for ventricular shunts, 4% for pacemakers and defibrillators, and about 40% for ventricular-assisted devices, data for device-related infections have been approximated. Native valve endocarditis (NVE) is an infection brought on by bacterial contact with the pulmonic and vascular endothelium of the heart. Streptococci, staphylococci,

gram-negative bacteria, and/or fungus illnesses are frequently to blame for this. In this situation, microbial cells can enter the bloodstream through the oropharynx, urinary tract, or gastrointestinal tract. As the intact valve endothelium gets damaged by the microorganisms that attach to it, even after the bacteria have been cleared by the immune system a non-bacterial thrombotic endocarditis (NBTE) develops at the injury location, as a result, a thrombus formation occurs, a condition where platelets, red blood cells, and fibrin are aggregated [2].

Infection	Organism
Urinary tract infection (UTI)	*Uropathogenic Escherichia coli (UPEC), Klebsilla pneumoniae, Enterococcus faecalis*
Lung infections (cystic fibrosis)	*Pseudomonas aeruginosa*
Otitis media	Nontypeable *Haemophilus influenzae*) and *Streptococcus pneumoniae*,
Endocarditis	VGS (Viridans group Streptococci), Staphylococcal spp.
Tonsillitis	Group A Streptococci (GAS)
Periodontitis	Gram-negative (GN) anaerobic bacteria
Necrotizing fasciitis	Group A Streptococci (GAS)
Osteomyelitis	Different bacterial species
Infectious kidney stones	GN bacilli

Figure 1: Disease related to bacterial infection: Diagramed showing the list of diseases related to the bacterial biofilms (WILEY).

The development of bio-film structures embedded in the gingival crevices may lead to pathological problems like chronic gingivitis or periodontitis. According to studies, persistent bacterial infections are the cause of nearly all types of periodontal diseases. In healthy individuals, oral biofilm mostly consists of Gram-positive facultative anaerobes (*Actinomyces naeslundiiand*MIRZAEIET *Streptococcus anginosus);* however, following chronic gingi-vitis or periodontitis, the number of Gram-negative anaero-bic bacteria (*Porphyromonas gingivalis, Aggregatibacteractinomycetemcomitans*,Bacteroides forsythus, *Prevotellaintermedia*,*Campylobacterrectus*,*Peptostreptococcusmicros*,and*Streptococcus intermedius*)and species of Prevotella, Eikenella, Fusobacterium, Capnocytophaga, Treponema, Veillonella, as well as other noncultivable Spi-rochetal bacteria, increases, and the bacterial count associ-ated with these complications are about times greaterthan those of similar species discovered in healthy individuals. The three periodontal bacteria that have the greatest scientific understanding are A. *actinomycetemcomitans*, *P. gingivalis*, and *B. forsythus*. These bacteria are capable of harboring a wide range of virulence factors, such as invasins and various proteases.

Proteases improve vascular permeability and decrease gingival crevicular fluid, which provides bacteria living on sub-gingival plaque with a rich source of micronutrients. The most important strains of periodontal bacteria are *P. gingivalis*, which can adhere to and invade epithelial cells in the human oral cavity. *Fusobacterium nucleatum* is an essential periodontal agent, notably linked to rapid and progressive periodontal disorders. Actinomyces *actinomycetemcomitans* are involved in periodontal disorders. Bacteroides forsythias is capable of entering human cells and inducing apoptosis in human cells. It contains many virulence determinants, such as those linked to polysaccharide formation and proteases. Capnocytophaga has been linked to both adolescent and adult periodontal diseases. This microorganism produces proinflammatory lipopolysaccharides and extracellular proteases that could disturb the secretory immunoglobulin A (sIgA).*Peptostreptococcus* micros is more common than 60% in individuals with advanced peri-odontitis, and it has also been linked to human dental implant failure.

When compared to healthy people, patients with periodontal problems are more likely to have spirochetal bacteria present. Two significant spirochetes, Trep-onema denticolaandTreponema vincentii, are also associ-ated with periodontal disorders that have the ability toproduce proinflammatory lipopolysaccharides and uncommon metabolic products, such as hydrogen sul-fide, ammonia, and indole which are toxic to the humancells. In addition to bacterial elements, genetic, behavioral, and physiological factors also play a role in the immunopathogenesis of periodontal diseases (Figure.2). Numerous people may be naturally predisposed to periodontal disorders, though this has not yet been proven. The hormonal changes brought on by puberty and childbearing can cause gingival enlargement. One of the factors that can raise the likelihood of periodontal disorders is smoking [3].

The most frequent genital tract infection in women during their reproductive years is bacterial vaginosis (BV), which has been linked to severe health issues like preterm delivery and the acquisition or transmission of a number of sexually transmitted diseases. *Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus spp*., *Bacteroides spp*., and *Prevotella spp*. are a few examples of the anaerobic bacteria that are significantly more prevalent in BV than the healthy lactobacilli that are typically present. BV etiology is still unknown due to its polymicrobial character. *G. vaginalis* is the main species in a thick vaginal multi-species biofilm, which is undoubtedly a factor in BV. Standard antibiotics, like metronidazole, are unable to completely eradicate the vaginal biofilm, which is similar to what occurs in many other biofilm-related infections and may account for the high recurrence rates of BV.

Furthermore, the beneficial vaginal microbiota can suffer from antibiotic therapy [4]. Patients with cystic fibrosis (CF) who have chronic *Pseudomonas aeruginosa* lung infections tend to have mucoid (alginate-producing) strains that develop biofilms. A biofilm is a well-organized bacterial community that is enclosed in a self-made polymer matrix made of polysaccharides, proteins, and DNA. Infective endocarditis (IE) continues to be linked with a high morbidity and mortality rate despite advancements in antimicrobial and surgical therapy. Bacterial biofilms on the endocardium, particularly on the aortic and mitral valves, which cause their deterioration, are a hallmark of IE.

Children frequently develop recurrent respiratory tract infections (RRTIs), which pose a significant problem to pediatricians. Bacterial biofilms have recently been linked to RRTIs and antibiotic resistance, which has caused significant concerns about how to treat recurrent middle ear infections, chronic rhinosinusitis, and pharyngotonsillitis. The goal of this chapter recent findings regarding potential treatment options for pediatric upper respiratory tract infections caused by biofilms. It concentrates on research in pediatric patients and the clinical consequences of recurrent disease. The study revealed that the connection between bacterial

biofilm and recurrent upper respiratory tract infections is a developing issue that could raise significant questions about infection management [5]. So, in this volume, we provide a summary of some infectious diseases brought on by biofilms.

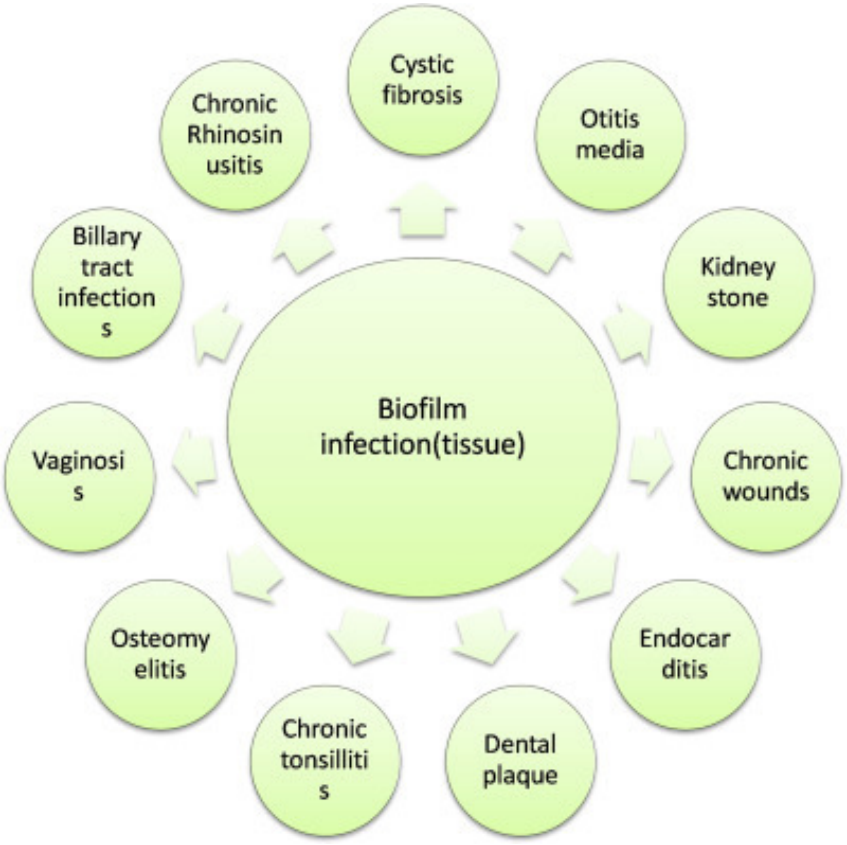

Figure 2: Biofilms infection: Diagramed showing the overview of the infectious disease caused by biofilms (Science direct).

LITURATURE SURVEY

Although heart disease and the various types of cancer are often considered the leading causes of death in industrialized nations, infectious diseases are comparable to or may even surpass them globally: Cardiovascular diseases caused 16.9 million fatalities in 2002, whereas cancer caused 7.9 million deaths. (WHO report 2004). The infectious diseases that cause human mortality have altered along with advancements in medicine and hygienic practices. Modern antibiotics and vaccines have successfully controlled acute infectious diseases brought on by specialized bacterial pathogens like diphtheria, tetanus, cholera, and plague, which were the leading causes of mortality at the start of the XXth century. Instead, commensal bacterial species that are found in the human body cause more than half of infectious diseases that impact patients with moderate immunosuppression; these bacterial species can cause chronic infections, are resistant to antimicrobial agents, and cannot be prevented by vaccines. Otitis media, native valve endocarditis, chronic urinary infections, bacterial prostatitis, osteomyelitis, and all infections associated with medical devices are a few examples of these diseases. Direct examination of the surface of medical equipment or tissues that have served as the site of persistent infections reveals the presence of numerous bacteria encased in a "biofilm," an exopolysaccharide matrix. Bacteria develop in the biofilm shielded from the effects of antibodies, phagocytic cells, and antimicrobial therapies. Candida albicans, a ubiquitous component of the human microflora and a significant human opportunistic fungal pathogen, is one of the human persistent infections we discuss in this

paper [2]. Infectious illnesses brought on by a variety of microorganisms, including *C. albicans*, can result from a disruption of the microbiome. Furthermore, it is believed that relationships between *C. albicans* and bacteria are important for maintaining human health. The primary biological trait of *C. albicans* that affects human health is its capacity to create biofilms. Particularly, the extracellular matrix (ECM) of the Candida biofilm performs a variety of roles, making it a highly desirable target for the treatment of infectious diseases linked to biofilms. Extracellular DNA (eDNA) also causes the morphological change from the yeast to the hyphal growth form during C. albicans biofilm development and plays a critical role in Candida biofilm formation and structural integrity. This review, which concentrates on pathogenic elements like eDNA in Candida biofilm development and the production of its ECM, offers valuable insight for future research to create a fresh approach to combat infectious diseases brought on by Candida formed biofilm [6].

It is known that bacteria can group together to form microbial colonies known as biofilms. Despite being historically thought of as environmental occurrences, bacterial biofilms are now frequently linked to human infections. The context of chronic infections that are resistant to current antibiotic regimens or infections that recur despite an acute reaction to treatment is where biofilms are most frequently invoked. Here, we examine the evidence that is currently accessible and its potential significance [7] for the role that biofilms play in infectious diseases.

Antibiotic resistance and the opportunistic *Stenotrophomonas maltophilia* pathogen's capacity to form biofilm make treating infections brought on by this condition in immunocompromised people challenging.*S. maltophilia* types can quickly cling to hospital surfaces and help spread the infection by producing biofilm. Additionally, the biofilm may lead to antibiotic tolerance, making some therapeutic choices ineffective and impeding the choice of an effective remedy. Traditional susceptibility tests do not yet provide treatment recommendations for infections linked with biofilms. Currently used chelating agents, natural and synthetic compounds, and widely prescribed antibiotics are used to control *S. maltophilia* biofilms. Biofilm susceptibility testing should incorporate both molecular and proteomic analyses as well as their characterization because biofilm age and matrix makeup influence the degree of antibiotic tolerance. As of right now, *S. maltophilia* infections caused by biofilm can be treated with several widely advised antibiotics [8].

In most bacterial diseases, biofilms are present. Collections of microorganisms known as biofilms are usually enclosed in a matrix made up of both bacterial and host materials. They develop on abiotic surfaces like contact lenses or intraocular lenses as well as native surfaces like heart valves. The biofilm matrix encourages bacterial adhesion to both other cells and flat surfaces. Thus, through coordinated multicellular behavior and huge 3-dimensional microbial communities with complicated architecture, biofilms are created. The architecture of the biofilm encourages the interchange of nutrients and waste. The use of implantable devices in medicine is significantly complicated by microbes' capacity to adhere to abiotic surfaces and develop in extremely stable communities. To create implantable devices and more potent antimicrobials that are immune to biofilms, a lot of work is currently being put into understanding the molecular makeup of biofilms [9].

In a self-generated extracellular matrix, surface-attached cells form microbial colonies known as biofilms. They have significant medical importance because they increase the spread of antimicrobial resistance and reduce susceptibility to antimicrobial agents. More and more, it is understood that bacterial and fungal microorganisms associated with biofilms contribute to a variety of infectious diseases, especially their persistence, and recurrence. In recent years, biofilms have also been linked to vaginal infections, including bacterial vaginosis (BV) and

vulvovaginal candidiasis (VVC), especially when treatment has failed and the infection has returned. This review's goal is to address how biofilms affect the management and treatment of BV and recurrent VVC and to draw attention to the need for more investigation into and creation of novel therapeutics that specifically target pathogenic vaginal biofilms [10].

Prosthetic joint infection (PJI) is still a serious issue. The number of PJI cases is also expected to increase, in line with the anticipated increase in joint replacement procedures. The development of biofilm by the pathogens responsible for causing PJI is essential for both its frequency and recalcitrance. The topic of microbial biofilms is becoming more popular, most likely as a result of the widespread recognition of their prevalence in natural, industrial, and clinical settings as well as the well-known difficulty in getting rid of them. In this overview, we go over the important problems with PJI and the difficulties with diagnosing and treating biofilms. We also go over cutting-edge methods for treating and preventing PJI caused by biofilms [11].

The oral microbiome, its interactions with our bodies, and how the community can impact our health, be protective, or result in the development of dental diseases have all been thoroughly and deeply understood over the past 100 years thanks to ground-breaking studies in oral microbiology. Concepts were established, theories were put forth, rejected, and later revisited from fresh perspectives during this exciting voyage. Dental plaque, which was once thought to be a polymicrobial community with generalized pathogenicity, is now understood to be a type of microbial biofilm with healthy, cariogenic, or periodontopathogenic profiles that develop as a consequence of particular ecological determinants and host-related factors. A more comprehensive understanding of a microbial community as the source of pathogenicity has taken the place of the "one pathogen, one disease" paradigm of oral infections. Modern technology is now able to investigate vast microbial communities linked to various clinical conditions, which has resulted in the discovery of several novel disease-associated species as well as possible pathobionts and pathobiomes. This enormous quantity of data accumulated over time has expanded our understanding of the causes of caries, periodontal, and peri-implant diseases and encouraged modernized approaches to their treatment and prevention [12].

CONCLUSION

For well over a century, acute infections brought on by pathogenic bacteria have been the subject of intensive research. Millions of people died from these infections in earlier centuries, but they have been successfully treated thanks to the advancement of contemporary vaccines, antibiotics, and infection control techniques. The majority of studies on bacterial pathogenesis have concentrated on acute infections, but a novel class of chronic infections brought on by bacteria growing in slime-enclosed aggregates known as biofilms has now been added to these diseases. Each year, millions of individuals in the developed world are affected by biofilm infections, which include pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis media, and implant- and catheter-associated infections. In general, during development and proliferation, bacteria have two living forms. Bacteria can be organized into sessile aggregates or can live as solitary, autonomous cells (planktonic) in both forms. The term "biofilm growth phenotype" is frequently used to describe the latter variety. Planktonic bacteria are thought to be involved in acute infections; these bacteria can usually be treated with antibiotics, but effective treatment relies on a prompt and accurate diagnosis. But when the bacteria manage to create a biofilm inside the human host, the illness frequently proves to be incurable and progresses into a chronic condition. Extreme tolerance to antibiotics and many other conventional antimicrobial agents, as well as an extreme ability to evade the host defenses, are crucial characteristics of chronic biofilm-based infections. In this

thesis, I will compile the most recent information on biofilms with a focus on chronic infections, recommendations for their diagnosis and management, and relate it to my earlier work on biofilms. It provides evidence to support the idea that the biofilm lifestyle, in which bacterial aggregation is the default mode, predominates in chronic bacterial infections and that the formation of the biofilm follows a pattern of adaptation to dietary and environmental factors. It creates a series of correlations to highlight the features of biofilms that, in my opinion, are most crucial and to see what can be inferred from previous decades of biofilm research. It attempts to make a connection between in vitro and in vivo research and suggests approaches for researching biofilms based on this understanding. This contrast how bacterial biofilms develop in environments with steady ecological conditions and how they emerge sporadically in environments with unstable ecological conditions, like infections. Although bacteria in both habitats live similarly (in biofilms), the struggle for dominance and survival is distinct. Hopefully, this chapter of the state-of-the-art ad my suggested recommendations will serve as the foundation and source of inspiration for additional study, better diagnostics, and effective treatments for existing and potential future biofilm infections

REFERENCES:

[1] R. M. Donlan, "Biofilms: Microbial life on surfaces," *Emerging Infectious Diseases*. 2002. doi: 10.3201/eid0809.020063.

[2] M. Jamal *et al.*, "Bacterial biofilm and associated infections," *Journal of the Chinese Medical Association*. 2018. doi: 10.1016/j.jcma.2017.07.012.

[3] R. Mirzaei *et al.*, "The biofilm-associated bacterial infections unrelated to indwelling devices," *IUBMB Life*, vol. 72, no. 7, pp. 1271–1285, 2020, doi: 10.1002/iub.2266.

[4] D. Machado, J. Castro, A. Palmeira-de-Oliveira, J. Martinez-de-Oliveira, and N. Cerca, "Bacterial vaginosis biofilms: Challenges to current therapies and emerging solutions," *Frontiers in Microbiology*. 2016. doi: 10.3389/fmicb.2015.01528.

[5] E. Nazzari, S. Torretta, L. Pignataro, P. Marchisio, and S. Esposito, "Role of biofilm in children with recurrent upper respiratory tract infections," *European Journal of Clinical Microbiology and Infectious Diseases*. 2015. doi: 10.1007/s10096-014-2261-1.

[6] K. Hirota, H. Yumoto, B. Sapaar, T. Matsuo, T. Ichikawa, and Y. Miyake, "Pathogenic factors in Candida biofilm-related infectious diseases," *Journal of Applied Microbiology*. 2017. doi: 10.1111/jam.13330.

[7] C. S. Hung and J. P. Henderson, "Emerging concepts of biofilms in infectious diseases.," *Mo. Med.*, 2009.

[8] S. Flores-Treviño, P. Bocanegra-Ibarias, A. Camacho-Ortiz, R. Morfín-Otero, H. A. Salazar-Sesatty, and E. Garza-González, "Stenotrophomonas maltophilia biofilm: its role in infectious diseases," *Expert Review of Anti-Infective Therapy*. 2019. doi: 10.1080/14787210.2019.1685875.

[9] I. Behlau and M. S. Gilmore, "Microbial biofilms in ophthalmology and infectious disease," *Archives of Ophthalmology*. 2008. doi: 10.1001/archopht.126.11.1572.

[10] C. A. Muzny and J. R. Schwebke, "Biofilms: An Underappreciated Mechanism of Treatment Failure and Recurrence in Vaginal Infections," *Clinical Infectious Diseases*. 2015. doi: 10.1093/cid/civ353.

[11] H. O. Gbejuade, A. M. Lovering, and J. C. Webb, "The role of microbial biofilms in prosthetic joint infections: A review," *Acta Orthopaedica*. 2015. doi: 10.3109/17453674.2014.966290.

[12] C. APV and T. ACR, "The Role of Bacterial Biofilms in Dental Caries and Periodontal and Peri-implant Diseases: A Historical Perspective.," *J. Dent. Res.*, 2019.

CHAPTER 18

ASPERGILLUS BIOFILMS ARE PRESENT IN NATURE AND IN THE IN VITRO CONDITION

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ABSTRACT:

A common filamentous fungus called Aspergillus that forms biofilms triggers invasive infections in patients with compromised immune systems. The mortality rate of invasive aspergillosis is still over 50% even with presently available antifungal medications, emphasizing the need for new treatments and a deeper comprehension of the virulence factors driving A. fumigatus pathogenesis. A. fumigatus switches to a biofilm mode of development during infection, where fungus hyphae are enclosed in a self-produced matrix. A. fumigatus needs galactosaminogalactan (GAG), a -1,4-linked linear exopolysaccharide of galactose (Gal) and N-acetylgalactosamine (GalNAc), to create its biofilms and is dependent on it for its virulence. Communities of adherent cells encased in an extracellular substance make up fungal biofilms. These biofilms are frequently discovered during infections brought on by several different fungal diseases. Due to their resilience to antifungals and host defenses, biofilm infections can be very challenging to treat clinically.

KEYWORDS:

Aspergillus Fumigatus, Aspergillus Biofilm, Aspergillus Conida, Filamentous Fungi, Fungal Biofilms.

INTRODUCTION

The most significant airborne fungal pathogen in the globe is Aspergillus fumigatus. All people inhale the conidia, which can result in a variety of illnesses ranging from simple rhinitis to deadly invasive aspergillosis (IA) in immunocompromised patients. In immunocompetent individuals with respiratory conditions like chronic obstructive pulmonary disease (22%), asthma (1–5%), and cystic fibrosis, as well as 15% allergic bronchopulmonary aspergillosis, the prevalence of A. fumigatus chronic infections, is steadily rising. Lung and sinus aspergillomas, as well as severe fungal keratitis infections, are also brought on by Aspergillus. Most investigations into the metabolism and virulence of Aspergillus were conducted with the fungus growing in fermentors or liquid flasks that were shaken until a few years ago. In order to conduct biochemical experiments and purify secreted molecules or antigens from culture filtrates or mycelial extracts, such an experimental setup was the most suitable.

In comparison, A. fumigatus grows as a colony that is defined by multicellular, multilayered hyphae that are embedded in an extracellular matrix in all Aspergillus infections as well as naturally on a solid substrate. (ECM). The definition of a biofilm, which is a structural microbial community of cells enclosed in an ECM, is consistent with the sort of growth that is being observed here. The biofilms of A. fumigatus, however, vary greatly from those of yeast. In this respect, septate hyphae that are structurally attached to form microbial colonies are present in the biofilms produced by filamentous fungi. Therefore, rather than using cells grown in the planktonic form in shaken flasks, a better knowledge of the infectious process should be based on the study of the biofilm colonies.

This will provide an overview of our current understanding of the biofilms created by A. fumigatus and the function played by ECM elements both in vivo and in vitro. We will also talk about A. fumigatus biofilms' molecular resilience to dangers from the outside [1]. Despite years of study on filamentous fungal development and growth in model fungi like Neurospora crassa and Aspergillus nidulans, and a recent rise in the understanding of the clinical significance of fungal biofilms, there are still a lot of unknowns regarding A. fumigatus biofilms. Clinicians face challenges when attempting to treat established A. fumigatus infections with fungal biofilms because the cellular processes orchestrating biofilm formation, structure, and function are still poorly understood.

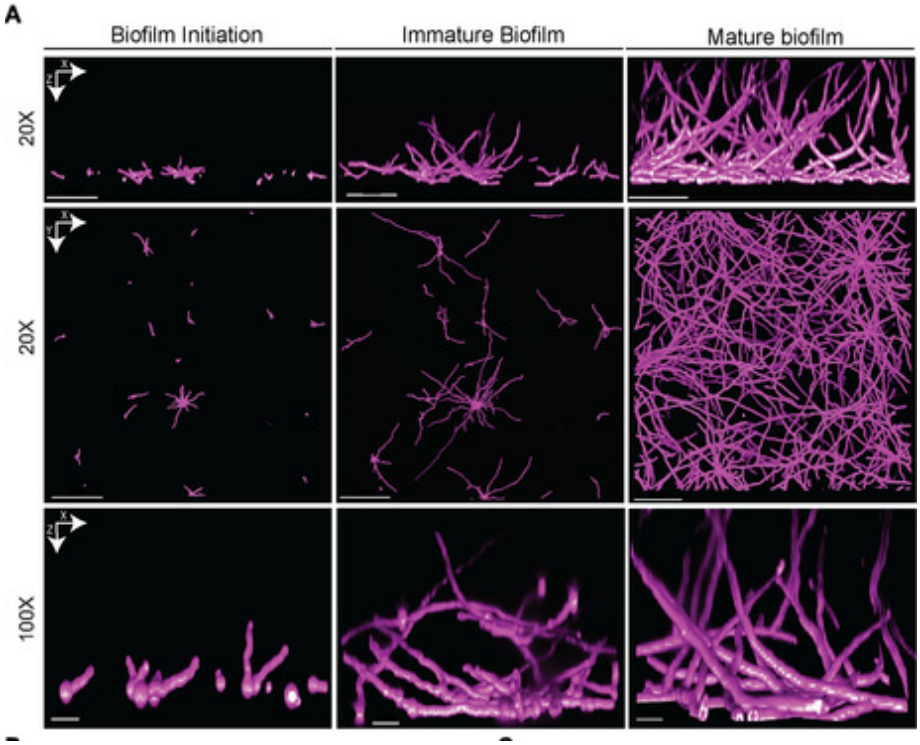

Figure 1: Aspergillus in the laboratory: Diagramed showing the growth of the Aspergillus in vitro condition (PLOS).

A population of A. fumigatus conidia passes through several developmental stages before forming biofilms. Emergent properties start to take on structure as the biofilm develops, and separate microenvironments are created therein. As a result, within a filamentous fungal biofilm, various hyphae, or even different sections of a single hypha, are in different physiological states. After inoculation of conidia, the first stage of A. fumigatus submerged biofilm formation occurs over the first 12 hours of culture; however, precise timing depends on the particular culture conditions. Under standard laboratory circumstances, 24- or 96-well polystyrene plates are used to grow the most popular submerged biofilm culture model (Figure.1). We have dubbed the initial 12-hour period of biofilm formation "biofilm initiation" in broad terms.

Unlike the initiation of many model bacterial biofilms where the transition from motile to a nonmotile state is a crucial defining step, the initiation of A. fumigatus biofilm formation is largely dependent on the conidia adhering to a surface and undergoing a series of developmental events that leads to the emergence of hyphae. Previous research has defined adhesion, swelling, and germination as separate and distinct stages of biofilm initiation; however, swelling and germination occur at a single-cell level rather than a community level, and adhesion is not necessarily restricted to a specific morphological stage of the fungus. A

high-order structure is mainly absent from the population at this point of initiation, there is little ECM secreted, and cells are still vulnerable to external stresses like antifungal drug therapy.

Only aspergilloma and IA have undergone ultrastructural investigations of Aspergillus biofilms. A. fumigatus develops in aspergillomas as a typical biofilm made up of hyphae that are firmly connected to an ECM. (Figure. 2A) Fungal keratitis has been linked to similar biofilms produced on contact lenses by filamentous fungus. In addition, bronchoalveolar lavages of patients with chronic pulmonary aspergillosis and neutropenic cancer patients with IA have both been reported to contain mycelial "grains" within biofilm formations (referred to as mycetoma). Despite growing independently in the lung, hyphae in IA typically have an ECM covering them. (Figure. 2B). Immunocytochemistry was used to determine the Aspergillus biofilms' ECM makeup after they had developed in mouse and human lung aspergillomas with IA. These investigations revealed the presence of galactomannan and GAG in the ECM of both lungs. It's interesting to note that while melanin and the polysaccharide 1,3 glucan were discovered in the ECM of aspergilloma biofilms, they were absent from IA and were only present in the inner layer of the hyphal cell wall.

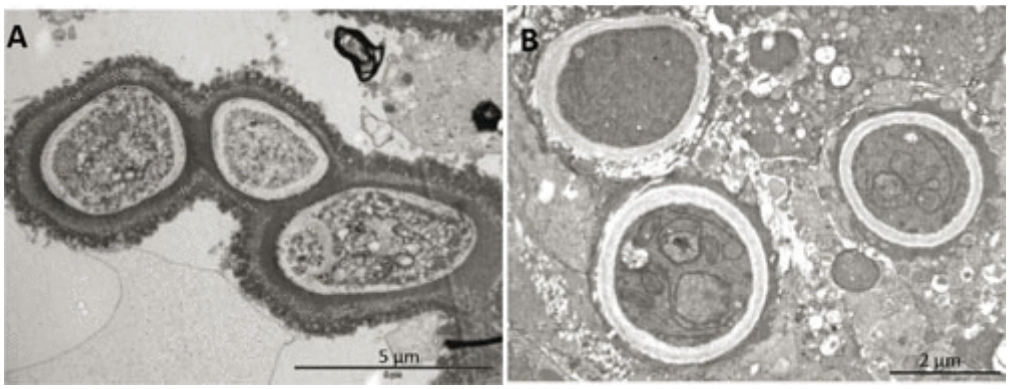

Figure 2: Aspergillus in nature; Diagramed showing the Aspergillus in vivo condition (ASM journal).

In the summary of this chapter, we discussed the presence of Aspergillus in nature and how it is different from the in vitro condition.

LITERATURE SURVEY

In the ecosystem, Aspergillus conida can be found everywhere, including in drinking water, freshwater, and bathing water. Aspergillosis is more likely to affect vulnerable people and those with allergic illnesses. Aspergillus should be avoided at all costs. There have been reports of possible aspergillosis outbreaks in hospital settings where the water source has been blamed. The danger of coming into contact with Aspergillus in water is not well understood. How does Aspergillus endure in liquid? Based on current research, this review examines the biofilm state of Aspergillus growth and contends that biofilms are to blame for Aspergillus' persistence in the water supplies of residential and healthcare institutions [2].

The most prevalent non-degradable solid refuse made of polyethylene is high-density polyethylene (HDPE). In the current research, different fungal strains that can break down HDPE were isolated from polyethylene waste dumped along the marine coast and tested in vitro. Two fungi strains known as VRKPT1 and VRKPT2 were discovered to be effective at degrading HDPE based on weight loss and FT-IR Spectrophotometric research. The isolated fungi were identified as Aspergillus tubingensis VRKPT1 and Aspergillus flavus VRKPT2 through the sequence study of ITS region homology. Even after a month of incubation, the

biofilm development seen under an epifluorescent microscope demonstrated the viability of fungal strains. Through SEM examination, the biodegradation of HDPE film nature was further examined. Because HDPE presents serious environmental risks, it has been demonstrated that fungal isolates can use virgin polyethylene as a carbon source without pre-treatment or pro-oxidant additives [3].

By primarily using submerged fermentation, Aspergillus is used for the commercial production of enzymes and organic acids. (SmF). Solid-state fermentation (SSF), as opposed to SmF, has several benefits. Although differences between SSF and SmF have been demonstrated, including reduced catabolite repression and substrate inhibition, as well as greater extracellular enzyme production, the underlying mechanisms are still unknown. The secretome of Aspergillus brasiliensis, which was obtained from cultures in a homogeneous physiological condition with high glucose concentrations, was examined to elucidate some differences between SSF and SmF. By raising the quantity of glucose, 74% of the regulated proteins made by SmF were downregulated, while all of those made by SSF were upregulated. Transaldolase was the protein that was most prevalent and elevated in SSF and that may also play a secondary role in fungal adhesion to the solid support. This study evidenced that SSF: (i) improves the kinetic parameters concerning SmF, (ii) prevents the catabolite repression, (iii) increases the branching level of hyphae and oxidative metabolism, as well as the concentration and diversity of secreted proteins, and (iv) favors the secretion of typically intracellular proteins that could be involved in fungal adhesion. The reason for all these variations is that molds are more tailored to growing in solid materials because doing so mimics their native environment[4].

In nature, light is a key signaling agent that controls fungi's secondary metabolites, morphogenetic processes, and physiological cycles. Light signaling transmits stress signals into the cell via the mitogen-activated protein kinase (MAPK) signaling system, exerting pressure on Aspergillus niger. Theoretical support for the use of light in the cultivation of filamentous fungi and other industrial uses will be provided by research on the impact of light on the A. niger biofilm. The effects of light signaling were here verified by the characterization of the A. niger biofilm under various light intensities. According to our findings, A. niger strongly accumulated protective mycelial melanin when exposed to light. We also found that light signaling activates the RlmA transcription factor in the MAPK signaling pathway, promoting the production of melanin, chitin, and other exopolysaccharides. We deleted the important melanin biosynthetic pathway genes Abr1 and Ayg1 because research on the significance of melanin to A. niger biofilm is uncommon. When melanin levels dropped in mutations, changes in hydrophobicity and electrostatic forces led to a reduction in biofilm [5].

Aspergillus fumigatus needs to adapt via genetic modifications or phenotypic plasticity to effectively infect or colonize human hosts or endure shifting environments. Based on the fungus' ability to create genetic variation, the genomic changes are made, and then the genotypes that are best suited to the new environment are chosen. The creation of biofilms, metabolic plasticity, and specific genetic changes that result in adaptations like host antifungal resistance have all received a lot of attention in science. The cyp51A gene is the target gene of the azoles, and recent scientific work has demonstrated improvements in understanding the natural relevance of parasex and how both asexual and sexual reproduction can lead to tandem repeat elongation. We will describe how the fungus can produce a genetic variation that can result in adaptation in this study. We will go over recent developments in our knowledge of A. fumigatus' life cycle to explain the variations in the rate and kind of

mutations that are produced in various habitats and how this can support adaptation, such as azole-resistance selection [6].

In industrial applications of aerobic fungal solid-state fermentation (SSF), oxygen transfer is a significant concern for two reasons: 1) Heat generation is inversely correlated with oxygen uptake, and it is well-known that heat removal is one of the major issues in scaled-up fermenters, and 2) Diffusion limitation may limit the oxygen supply to the mycelium on the surface or inside the substrate ppapers. The first experimental proof that aerial hyphae are crucial for fungal respiration in SSF is provided in this paper. Aerial hyphae made up to 75% of the oxygen absorption rate in A. oryzae cultures on a model substrate made of wheat flour. This is because A. oryzae produces a lot of aerial mycelium, and oxygen diffuses quickly through the aerial hyphae layer's gas-filled openings. Diffusion limitation is much less significant for A. oryzae than it was originally thought to be for R. oligosporus and C. minitans in the densely packed mycelium layer that forms closer to the substrate surface and has liquid-filled pores. Additionally, it implies that A. oryzae's overall oxygen uptake rate is significantly higher than what R. oligosporus and C. minitans' tightly packed mycelium layer would predict. This would suggest that refrigeration issues exacerbate. Thus, it is crucial to define the metabolic function of aerial hyphae in SSF [7].

A wide variety of bioactive substances produced by actinomycetes and filamentous fungi have used as antimicrobials, anticancer agents, or agrochemicals. They have far more gene clusters for natural products in their genomes than was initially thought, and new strategies are needed to tap into this source of possible new medicines.

Here, we demonstrate that the filamentous model microorganisms Streptomyces coelicolor and Aspergillus niger's secondary metabolism is significantly affected by co-cultivation. The cyclic dipeptide cyclo(Phe-Phe) and 2-hydroxyphenylacetic acid, both of which were produced by A. niger in response to S. coelicolor, were two substances that particularly correlated to co-cultures that were discovered through NMR-based metabolomics and multivariate data analysis. The novel substances (E)-2-(3-hydroxyprop-1-en-1-yl)-phenol and (2E,4E)-3-(2- carboxy-1-hydroxyethyl)-2,4-hexadienedioxic acid, respectively, were produced as a consequence of biotransformation studies with o-coumaric acid and caffeic acid. This demonstrates the effectiveness of microbial co-cultivation along with NMR-based metabolomics as a pathway for finding new natural products [8].

Saprotrophic Aspergillus fumigatus is a fungus that primarily lives in dirt. The fungus has developed the ability to adjust to and flourish in hostile settings as a result of its ecological niche.

This ability has enabled the fungus to withstand and outlive the host defenses of humans and, in addition, to cause one of the most severe lung illnesses in terms of morbidity and mortality. In this review, we will provide (i) a description of the biological cycle of A. fumigatus; (ii) a historical perspective of the spectrum of aspergillus disease and the current epidemiological status of these infections; (iii) an analysis of the modes of the immune response against Aspergillus in immunocompetent and immunocompromised patients; (iv) an understanding of the pathways responsible for fungal virulence and their host molecular targets, with a specific focus on the cell wall; (v) the current status of the diagnosis of different clinical syndromes; and (vi) an overview of the available antifungal armamentarium and the therapeutic strategies in the clinical context. We have also been able to redefine the opportunistic pathogenesis of A. fumigatus thanks to the development of new ideas like nutritional immunity and the integration and rewiring of numerous fungal metabolic activities happening during lung invasion [9].

CONCLUSION

Due to their resistance to antifungal medications, biofilms can develop into a troublesome clinical entity. The majority of the evidence for how Aspergillus biofilms enable the fungus to withstand external dangers was gathered in vitro. Verifying the in vitro findings on A. fumigatus biofilms in vivo will be the next stage. Although there are currently very few in vivo models, many are being researched. They involve in vivo animal models such as an A. fumigatus biofilm diffusion chamber implanted subcutaneously in a mouse and a murine model of contact lens–associated fungal keratitis and ex vivo models such as human primary bronchial epithelial cells at the air-liquid interface and the human airway epithelium. These models will be the most helpful for comprehending how a biofilm forms inside a patient and for examining how the host reacts as an A. fumigatus biofilm develops. The efficacy of novel therapeutic approaches, particularly combinatorial medicines, will also be evaluated using these ex vivo and in vivo models. Although they cause ROS buildup in the fungal cell, ROS-inducing antimicrobial drugs (lactoferrin, defensins, the antimicrobial peptides arenicin and pleurocidin, derivatives of 2-aminotetralin, etc.) are not effective against the pathogen directly.

They do, however, show synergistic interactions with the echinocandins, polyenes, and azoles, which are presently used as antifungal agents. Anti-inflammatory medications like diclofenac and ibuprofen work in concert with amphotericin B or echinocandins to inhibit the growth of Candida spp. biofilm. When combined with echinocandins, the Hsp90 inhibitor geldanamycin significantly slows down the formation of biofilm in A. fumigatus. Studying the A. fumigatus biofilm in vivo and its microbiome is another necessity. It has not yet been documented that typical commensal and pathogenic flora inhabiting the respiratory system can create a microbiota lung biofilm.

The two most prevalent bacterial and fungal residents of the lung microbiota are P. aeruginosa and A. fumigatus. P. aeruginosa infects 80% of cystic fibrosis patients, and A. fumigatus colonizes 60% of them as well. Because P. aeruginosa colonization under biofilm structures occurs sooner than A. fumigatus infections, it is suggested that these conditions favor A. fumigatus infections. According to data, P. aeruginosa initially creates toxins that prevent fungal development but later changes the fungus' physiology to make it more resilient to stress. However, P. aeruginosa forms biofilms in response to A. fumigatus secondary compounds. We are presently developing an experimental murine model that will enable us to research chronic bacterial and fungal co-infections. Our knowledge of the function of the A. fumigatus biofilm during lung invasion will become even more complex as a result of microbiota.

REFERENCES:

[1] A. Beauvais and J.-P. Latgé, " Aspergillus Biofilm In Vitro and In Vivo ," *Microbiol. Spectr.*, 2015, doi: 10.1128/microbiolspec.mb-0017-2015.

[2] M. Richardson and R. Rautemaa-Richardson, "Exposure to aspergillus in home and healthcare facilities' water environments: Focus on biofilms," *Microorganisms*. 2019. doi: 10.3390/microorganisms7010007.

[3] R. Sangeetha Devi, V. Rajesh Kannan, D. Nivas, K. Kannan, S. Chandru, and A. Robert Antony, "Biodegradation of HDPE by Aspergillus spp. from marine ecosystem of Gulf of Mannar, India," *Mar. Pollut. Bull.*, 2015, doi: 10.1016/j.marpolbul.2015.05.050.

[4] D. Salgado-Bautista, T. Volke-Sepúlveda, F. Figueroa-Martínez, U. Carrasco-Navarro, A. Chagolla-López, and E. Favela-Torres, "Solid-state fermentation increases secretome complexity in Aspergillus brasiliensis," *Fungal Biol.*, 2020, doi: 10.1016/j.funbio.2020.04.006.

[5] W. Sun *et al.*, "Light signaling regulates aspergillus niger biofilm formation by affecting melanin and extracellular polysaccharide biosynthesis," *MBio*, 2021, doi: 10.1128/mBio.03434-20.

[6] J. Zhang, A. J. M. Debets, P. E. Verweij, and E. Snelders, "Azole-resistance development; how the Aspergillus fumigatus lifecycle defines the potential for adaptation," *Journal of Fungi*. 2021. doi: 10.3390/jof7080599.

[7] Y. S. P. Rahardjo, F. J. Weber, E. Paul Le Comte, J. Tramper, and A. Rinzema, "Contribution of aerial hyphae of Aspergillus oryzae to respiration in a model solid-state fermentation system," *Biotechnol. Bioeng.*, 2002, doi: 10.1002/bit.10222.

[8] C. Wu, B. Zacchetti, A. F. J. Ram, G. P. Van Wezel, D. Claessen, and Y. H. Choi, "Expanding the chemical space for natural products by Aspergillus-Streptomyces co-cultivation and biotransformation," *Sci. Rep.*, 2015, doi: 10.1038/srep10868.

[9] J. P. Latgé and G. Chamilos, "Aspergillus fumigatus and aspergillosis in 2019," *Clin. Microbiol. Rev.*, 2020, doi: 10.1128/CMR.00140-18.

CHAPTER 19

PREBIOTICS AN EFFECTIVE AGENT THE PATHOGENIC BIOFILMS

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ABSTRACT:

Bacterial populations can develop into biofilms and can be found in a wide range of natural and human-associated habitats. Microorganisms embedded in a matrix made of polysaccharides, proteins, and nucleic acids are largely responsible for pathological chronic conditions, and all the bacterial infections linked to implanted medical devices or prosthetics. The typical traits of biofilm infections include a slow onset, mild symptoms, a propensity for chronicity, and a refractory reaction to antibiotic treatment. Effective strategies to combat biofilms are still needed even though the molecular processes underlying host defenses and resistance to antimicrobial agents have been thoroughly explained. Probiotics containing lactic acid bacteria (LAB) are proving to be effective tools for controlling pathogen overgrowth, preventing adhesion, and preventing the development of biofilms. Therefore, using probiotics or their metabolites to inhibit biofilm formation and stability, quench and disrupt bacterial communication and aggregation, and interfere with these processes may represent a new frontier in clinical microbiology and a viable option for antibiotic therapies. This study provides an overview of the state-of-the-art regarding the experimental and therapeutic uses of LAB to prevent the growth of pathogenic biofilms or to interfere with their stability.

KEYWORDS:

Anti-Biofilm, Biofilm Growth, Lactic Acid, Probiotic Strains, Pathogenic Biofilm.

INTRODUCTION

One of the major issues of the antibiotic era is pathogenic bacterial biofilms. A biofilm is a collection of microorganisms and the extracellular substances they generate that adhere to biotic or abiotic surfaces and are distinguished by highly specialized interactions [3]. Extracellular polymeric substances (EPS), which make up the matrix of the self-produced slime, are embedded with bacteria that create biofilms. This growing state can change the biological and physiological properties of bacteria, including their capacity for reproduction, growth, gene transcription, and antibiotic tolerance. Schematically, the formation of a differentiated biofilm requires five maturation stages: (i) initial attachment of planktonic bacteria (reversible) to a surface; (ii) production and secretion of EPS and/or other means of docking, and specific adhesins (e.g., flagella, autotransporter proteins, fimbriae, curli fibers, and F-type conjugative pilus) that drive the transitional attachment from reversible to irreversible ; (iii) early-maturing of biofilm architecture as a super cellular structure; (iv) late-maturing of micro-colonies and evolution into a mature biofilm; and (v) detachment of cells from the biofilm and dispersion into the surrounding environment (Figure 1).

All of these processes are tightly controlled by various cell-to-cell communication molecules that are in charge of population density-dependent gene expression, which has a significant impact on the biofilm formation process. Bacterial survival and growth are made possible by the creation of the EPS matrix, which is made up of polysaccharides, proteins, and nucleic

acids (extracellular DNA—eDNA). This protected niche provides a steady supply of nutrients as well as defense from the host immune system, disinfectants, and antibiotics. With the help of biofilms, bacteria can evade phagocytosis and immune recognition while expressing genetic switches (or response regulators) that interfere with immune cell activity. Around the globe, biofilms and/or bacteria that are resistant to antibiotics are responsible for up to 80% of chronic infections. Compared to their planktonic counterparts, microbes growing in biofilms can be 100–1000 times more drug-resistant [1].

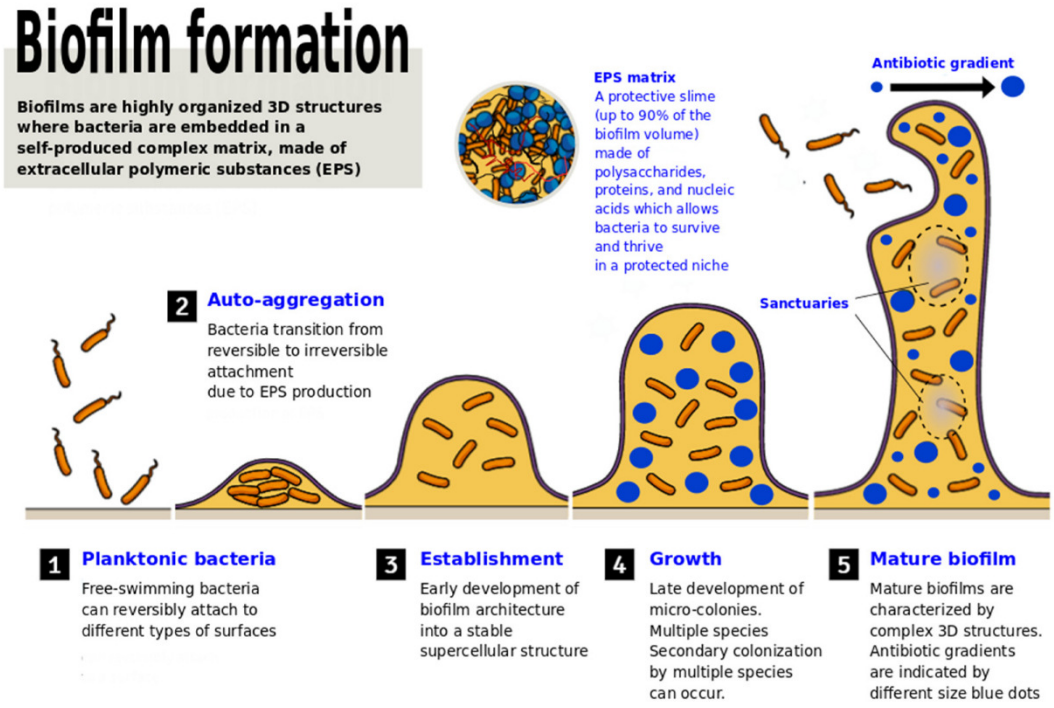

Figure 1: Biofilm development: Diagramed showing the different stages involved in pathogenic biofilm development (MDPI).

The creation of fresh biofilm-fighting tactics would be helpful in the clinic due to the shortcomings of well-known approaches. Recent data suggest that probiotics have created new opportunities for combating pathogenic biofilms. Probiotics are an excellent choice for new anti-virulence agents because they do not exert the same powerful selective pressure on resistant isolates as conventional antibiotics do and because they are less cytotoxic than QS-suppressing agents. Probiotics can reduce the activity of pathogenic bacteria and their adherence to surfaces through a variety of processes. Additionally, they interfere with biofilm integrity/quality, inhibit QS, prevent biofilm formation and the survival of biofilm pathogens, and ultimately result in the eradication of biofilms.

Some of these molecular mechanisms include the secretion of antagonistic substances (e.g., surfactants, bacteriocins, exopolysaccharides (EPS), organic acids, lactic acid, fatty acids, enzymes (amylase, lipase) and hydrogen peroxide) and the generation of unfavorable environmental conditions for pathogens (e.g., pH alteration as well as competition for surface and nutrients), (Figure 2). The competitive adhesion of probiotics to human tissues or medical equipment stops harmful bacteria from colonizing. Probiotics also inhibit the development of pathogenic biofilms by lowering environmental pH, indole production (a signal molecule in QS), and biofilm biomass. The probiotic strains can be isolated from a variety of sources, including foods, the atmosphere, plants, animals, and people.

They can then be recognized and classified using genetic, biochemical, and microbiological methods. *L. rhamnosus, S. oralis, Streptococcus salivarius, L. fermentum,* and *L. plantarum* The most frequently recorded probiotic strains with anti-biofilm activity are *L. casei, L. acidophilus, L. brevis, L. sporogenes, L. salivarius, L. delbrueckii, L. pentosus, Bifidobacterium lactis*, and *B. longum*. The development of several in vitro biofilm models involved attaching microorganisms to adhesive surfaces.20 These models all lack elements of the environment and host immune capability. Since it is virtually impossible to study the development of infectious diseases in humans, animal models are taken into consideration. A rabbit model of ischaemic and infected wounds and an MRSA rodent model22 were created. Additionally, a removable in vivo abutment that resembled tooth implants were created. A new human plasma biofilm model was created to study the effects of probiotics on pathogens and replicate a biofilm-challenged human wound environment to address issues both *in vitro* and in vivo. *Bifidobacterium bifidum* BGN4 is a probiotic strain of the Bifidobacterium that has been a main component of nutraceutical goods for the past 20 years. *B. bifidum* BGN4 has been characterized and demonstrated in vitro (i.e., phytochemical bio-catalysis, cell adhesion, anti-carcinogenic effects on cell lines, and immunomodulatory effects on immune cells) and in vivo experiments for its different bio-functional effects and potential for industrial application.

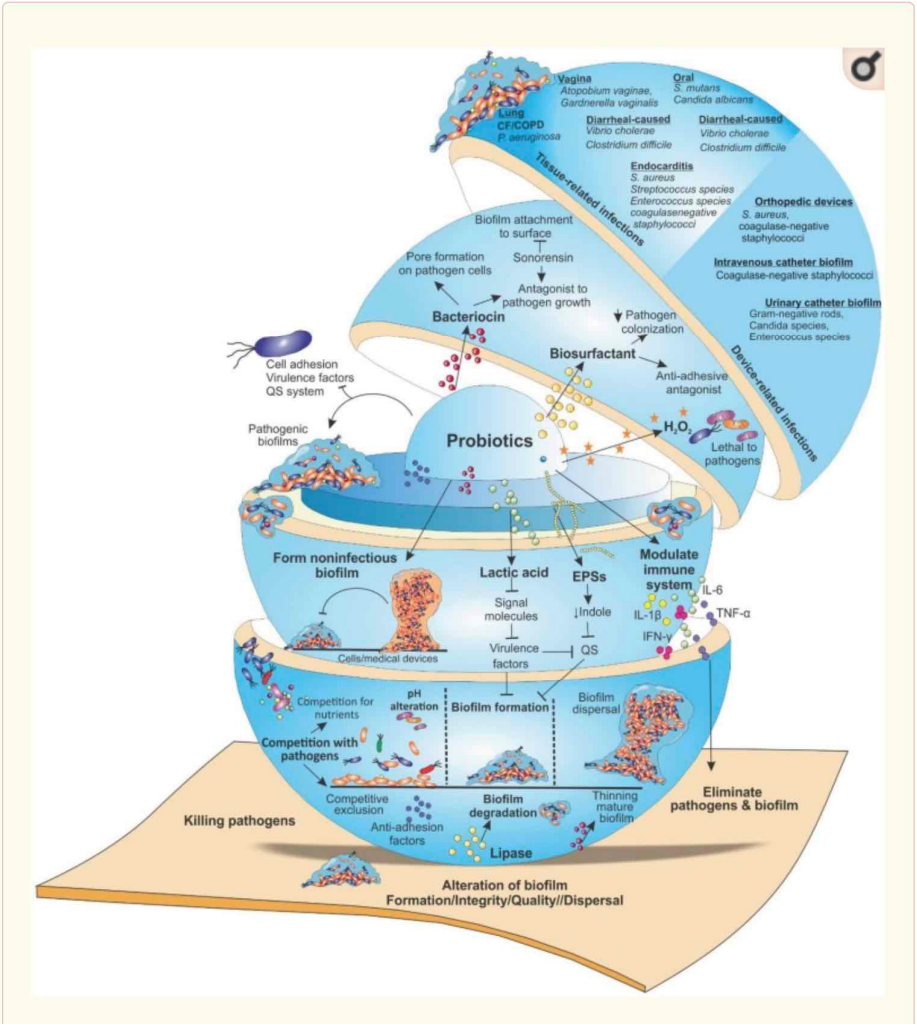

Figure 2: Probiotic: Diagramed showing the different probiotics' role against the pathogen (siani probiotic body).

LITERATURE SURVEY

Less research has been done on Bifidobacteria's capacity to contrast pathogenic biofilms than has been done on lactobacilli. Lower effectiveness compared to other LAB has also been noted in some experimental works. For instance, Miyazaki et al. (2010) emphasized that CS of a Lactobacillus strain does not affect Bifidobacteria while having a strong bactericidal effect on auto-aggregative *E. coli*. Bifidobacteria experiments with animal models have also produced inconsistent findings in the lab. For instance, *S. aureus* 8325-4 strain was shown to be sensitive to L. acidophilus in vitro, while *B. bifidum* inhibited the same bacteria's ability to induce experimental intravaginal *staphylococco*sis in mice the best. In vivo studies have shown a broad range of positive effects for *B. bifidum* BGN4, including suppressed allergic responses in mouse models and anti-inflammatory bowel disease. Clinical studies have also shown eczema in infants and adults with irritable bowel syndrome. Chronic infections, infections caused by malfunctioning medical equipment, and infections caused by biofilms have all been significant therapeutic issues. Biofilms pose a global danger to human health because they are challenging to eradicate and control and are not completely accessible to the human immune system and antibiotics. There are ways to combat biofilms, most of which center on preventing their adhesion and development. Nowadays, there is increasing interest in using probiotics and their derivatives to combat pathogenic biofilms. In this review, we take a close look at probiotics with the end goal of preventing the development and maturation of biofilms. In general, it would be prudent to gain more understanding of the processes by which probiotics and their derivatives can be used to treat biofilm infections [2].

Bioactive substances are emerging as novel biocontrol agents to prevent pathogens from forming biofilms due to the variety of their functional properties. Exopolysaccharide (EPS) isolated from *Lactobacillus plantarum* (EPLB) was physicochemical characterized in this research, and it's in vitro impact on biofilm formation was investigated. The polydispersity index of the EPS, which was calculated to be 1.2, was 36 kDa. The tested EPLB demonstrated antibiofilm effect concentration based on Gram-positive and negative strains, with a Minimal Inhibition Concentration (MIC) values varying between 1 mg/ml and 10 mg/ml. Two out of the four pathogenic strains looked to have more than 50% of their biofilm development inhibited by the EPS. The EPS's capacity to affect how biological membranes work, such as hydrophobicity, which decreased (P 0.05) when the EPS was used at a concentration of 512 g/ml, maybe the cause of the antibiofilm activity. With a percentage of 64% and 66%, respectively, this EPS, which had no cytotoxic effects, demonstrated an antioxidant impact on the quenching of DPPH radicals and the inhibition of lipid peroxidation. When these biological characteristics are examined collectively, EPLB can be thought of as a potential prebiotic agent in the development of fresh treatment plans for infections caused by bacterial biofilms [3].

Vibrio cholerae, which causes diarrhea, is prevalent in developing nations like India and is linked to a high mortality rate, particularly in children. On the gut epithelium, *V. cholerae* is known to produce biofilms that, once established, are resistant to the effects of antibiotics. Agents that disperse already-formed biofilms and inhibit their formation are therefore thought to have therapeutic advantages. The use of antibiotics to treat cholera is linked to adverse reactions like gut dysbiosis brought on by the loss of gut flora and the growing issue of drug resistance. Therefore, the hunt for secure substitute therapeutic agents is necessary. Here, using an in vitro assay, we tested the lactobacilli spp. isolated from the feces of healthy children for their capacity to inhibit the formation of biofilms and disseminate the preformed biofilms of *Vibrio cholerae* and *Vibrio parahaemolyticus*. The findings demonstrated that all

seven isolates of Lactobacillus spp. used in the study's culture supernatant (CS) suppressed the development of V. cholerae's biofilm by more than 90%.

Although pH neutralization of CS had only minor effects on their biofilm inhibitory potential, it eliminated their antimicrobial activities against V. cholera. Additionally, all of the lactobacilli isolates' CS caused 62–85% of the preformed *V. cholerae* biofilms to disperse; however, pH neutralization of the CS decreased the ability for 4 out of 7 isolates to disperse biofilms by 19–57%. The studies also revealed that none of the lactobacilli isolates' CS had antimicrobial action against *V. parahaemolyticus*, but five of the seven isolates inhibited the development of its biofilm by 62–82%. But none of the CS managed to break up the biofilms that *V. parahaemolyticus* had already developed. It was also established whether CS could prevent Vibrio spp. from adhering to the epithelium cell line. Thus, we conclude that pH is a factor in the strain-specific biofilm dispersive activity of lactobacilli CS. Probiotic strains with dispersive action at high pH may have greater therapeutic potential because Vibrio is known to form biofilms in the intestinal niche with physiological pH in the range of 6-7[4].

As an alternative and ecologically friendly candidate to control microbial pathogens, probiotics hold a lot of promise. Based on their anti-listerial action, six isolated lactic acid bacteria (LAB) were selected in this instance. The 16S rRNA gene was used to identify anti-listerial LAB strains. Listeria monocytogenes biofilm inhibition assays on stainless-steel coupons (SS), lettuce, and a minimal biofilm eradication concentration (MBECTM) biofilm device was used to assess the anti-listerial activities of these isolates. The results showed that L. monocytogenes biofilm cells were suppressed by up to 2.17 log CFU/cm2, 1.62 logs CFU/cm2, and 1.09 log CFU/peg on SS, lettuce, and MBECTM, respectively, after co-culture with LAB for 24 hours. Although these LAB bacteria prevented the growth of L. monocytogenes biofilms on both surfaces, the impact on lettuce surfaces was less effective than it was on SS. These findings support the possible application of LAB strains to prevent pathogenic bacteria from forming biofilms on vegetable products and in the food industry [5].

Listeria monocytogenes, which can form biofilms, can be extremely contagious and challenging to eradicate in the food business. In this investigation, the anti-biofilm properties of *Saccharomyces cerevisiae* CFS against *L. monocytogenes* were examined. Exopolysaccharides (EPS) production, exopolysaccharide gene expression, morphological changes, and cell surface properties (auto-aggregation and cell surface hydrophobicity) were studied to explore the anti-biofilm mechanism. The CFS prevented and eliminated *L. monocytogenes'* biofilm. All L. monocytogenes strains treated with CFS showed a substantial reduction in auto-aggregation, cell surface hydrophobicity, and EPS production. Following CFS treatment, real-time polymerase chain reaction showed significant downregulation of virulence factors (prfA and hlyA) and genes linked to biofilm. Scanner electron microscopy and confocal laser scanning microscopy were also used to demonstrate the anti-biofilm effects of CFS against L. monocytogenes. Thus, it was established that L is resistant to the anti-biofilm effects of the CFS of S. cerevisiae isolated from *cucumber jangajji* [6].

In this research, three strains of *C. albicans*—two clinical strains and one reference strain—were directly inhibited by isolated Lactobacillus strains from caries-free subjects. Thirty Lactobacillus strains were separated, and their antimicrobial efficacy against *C. albicans* biofilms in vitro was assessed. The strains of *L. paracasei* 28.4, *L. rhamnosus* 5.2, and *L. fermentum* 20.4 showed the most notable inhibition of *C. albicans*. The growth of biofilm was inhibited and the emergence of hyphae was postponed by the co-incubation of these microorganisms. The expression of *C. albicans* biofilm-specific genes was downregulated, which was used to identify the obstruction to biofilm formation. (ALS3, HWP1, EFG1 and

CPH1). Through the inhibition of *C. albicans* biofilms, *L. paracasei* 28.4, *L. rhamnosus* 5.2, and *L. fermentum* 20.4 showed their capacity to exhibit antifungal activity [7].

Probiotic bifidobacterial and lactobacillus lectin preparations had system affinity for mannan and polysaccharides of the mucin-type. It was established that these lectins have antifungal and antifungicidal effects on clinical isolates of *Candida albicans* that are nystatin-resistant. Concerning *C. albicans* and *Staphylococcus aureus* biofilms, lectins showed destructive properties based on the clinical strain's origin and the lectin preparation method. It was discovered that lectins and nystatin have complementary antipathogen actions. Pathogen biofilm degradation in the presence of lectins took place in a series of stages, including biofilm refinement, the emergence of edge cavities, segmentation, detachment of pieces, and their lysis. Compared to staphylococci, fungi had a more complicated reaction to lectins. Pictures of lectin antipathogen activity were enhanced by cold stress. According to the data, pathogen biofilm destroyers are a novel class of antimicrobials that includes probiotic bacterial lectins [8].

CONCLUSION

Data on antibiofilm activity on different respiratory, genito-urinary, wound, and tissue pathogens is beginning to convince experts that certain probiotic combinations are useful in the human field. However, there is still a way to go, particularly in terms of their regular in vivo use. The information presented here should promote further research into probiotic-biofilm interactions and ways to combat biofilm diseases using so-called "good bacteria" like bifidobacteria and lactobacilli, while also emphasizing the existence of "useful" or "good" biofilms. We must concentrate our study on the creation of such promising strains because mechanisms of action and antibiofilm activities must be viewed as strain-related. Although it is still too early to tell, given the questionable longevity of antibiotics, it would be advised to investigate alternative means, and so far, probiotics represent one of the most promising. It is frequently disputed whether probiotics will become widely used drugs or medicaments in the future.

REFERENCES:

[1] G. Meroni, S. Panelli, G. Zuccotti, C. Bandi, L. Drago, and D. Pistone, “Probiotics as Therapeutic Tools against Pathogenic Biofilms: Have We Found the Perfect Weapon?,” *Microbiol. Res. (Pavia).*, 2021, doi: 10.3390/microbiolres12040068.

[2] A. Barzegari *et al.*, “The battle of probiotics and their derivatives against biofilms,” *Infection and Drug Resistance*. 2020. doi: 10.2147/IDR.S232982.

[3] A. Mahdhi *et al.*, “Extracellular polysaccharide derived from potential probiotic strain with antioxidant and antibacterial activities as a prebiotic agent to control pathogenic bacterial biofilm formation,” *Microb. Pathog.*, 2017, doi: 10.1016/j.micpath.2017.05.046.

[4] S. Kaur, P. Sharma, N. Kalia, J. Singh, and S. Kaur, “Anti-biofilm properties of the fecal probiotic Lactobacilli against Vibrio spp,” *Front. Cell. Infect. Microbiol.*, 2018, doi: 10.3389/fcimb.2018.00120.

[5] M. I. Hossain *et al.*, “Inhibitory effects of probiotic potential lactic acid bacteria isolated from kimchi against Listeria monocytogenes biofilm on lettuce, stainless-steel surfaces, and MBEC^{TM} biofilm device,” *LWT*, 2020, doi: 10.1016/j.lwt.2019.108864.

[6] Y. J. Kim, H. H. Yu, Y. J. Song, Y. J. Park, N. K. Lee, and H. D. Paik, “Anti-biofilm

effect of the cell-free supernatant of probiotic Saccharomyces cerevisiae against Listeria monocytogenes," *Food Control*, 2021, doi: 10.1016/j.foodcont.2020.107667.

[7] R. D. Rossoni *et al.*, "Antifungal activity of clinical Lactobacillus strains against Candida albicans biofilms: identification of potential probiotic candidates to prevent oral candidiasis," *Biofouling*, 2018, doi: 10.1080/08927014.2018.1425402.

[8] M. Lakhtin, V. Alyoshkin, V. Lakhtin, S. Afanasyev, L. Pozhalostina, and V. Pospelova, "Probiotic Lactobacillus and Bifidobacterial Lectins Against Candida albicans and Staphylococcus aureus Clinical Strains: New Class of the Pathogen Biofilm Destructors," *Probiotics Antimicrob. Proteins*, 2010, doi: 10.1007/s12602-010-9046-3.

CHAPTER 20

THE WAY OF COMPETITION IN COLONIES OF BIOFILMS

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ABSTRACT:

Since Antonie van Leeuwenhoek first described these communities in the late 1600s, our understanding of the nature and evolution of microbial biofilms has considerably expanded. However, the majority of biofilm studies focus on mono-species cultures, while in nature, the majority of biofilm communities are made up of a diversity of microorganisms. The types of microorganisms that make up a mixed biofilm and their relationships have a significant impact on how the community develops and takes on its characteristic form. We concentrate on interactions within a multi-species biofilm and how they affect the makeup of the mixed community in this study. Interspecies interactions usually involve quorum sensing for communication and either metabolic cooperation or competition. Within a biofilm, species may interact antagonistically inhibiting development and competing for nutrients, for example or cooperatively. The latter can lead to the emergence of several advantageous forms. These include co-aggregation's promotion of biofilm development, metabolic cooperation in which one species makes use of a metabolite produced by a nearby species, and enhanced resistance to antibiotics or host immune responses in comparison to mono-species biofilms. Mixed biofilms exhibit advantageous interactions that have significant consequences for the environment, business, and medicine. For instance, the latter affects how biofilm-related infections, like those that appear in the lungs of people with cystic fibrosis, develop and are treated.

KEYWORDS:

Biofilm Communities, Ecological Competition, Kin Recognition, Microbial Communities, Reducing Bacteria.

INTRODUCTION

The accumulated microbial communities that are adhered to either natural or man-made surfaces are known as biofilms. Extracellular polymeric substance (EPS), which is composed of extracellular polysaccharides, cellular debris, DNAs, and proteins, surrounds this mono- or polymicrobial aggregate to enhance microbial adhesion and promote the development of microcolonies. EPS makes up 75–95% of the volume of a developed bacterial biofilm while bacteria only take up 5-25% of the space. In contrast to planktonic bacteria, which are adapted to harsh environments, biofilm-forming bacteria benefit from harsh environments and experience rapid development. It is challenging to eradicate these microbes because bacteria within biofilms are ten- to ten-fold more tolerant and resistant to antibiotic therapy than their free-swimming counterparts. According to the National Institutes of Health (NIH) in the U.S., biofilms are thought to be responsible for about 75% of human microbial illnesses.

The numerous negative effects that biofilms have on human health have been compiled in several reviews. Cystic fibrosis, periodontitis, infective endocarditis, and persistent wounds are common illnesses brought on by bacterial biofilms. Chronic wounds that have developed biofilms cause protracted inflammatory responses against infectious microbes and slow

wound healing. Additionally, biofilms that develop on the surfaces of medical devices like mechanical heart valves, catheters, contact lenses, and dental implants can cause serious bacteremia and persistent internal infections that can harm human systems. Additionally, the presence of biofilms in the food industry increases the risk of foodborne outbreaks among consumers or employees, endangering both human health and economic growth. In water distribution networks, microorganisms primarily proliferate by creating biofilms. Human health could be harmed and waterborne illnesses could be brought on by drinking water contaminated with pathogenic microbes. It is crucial to investigate practical ways to prevent the spread of biofilms, which are pervasive in modern society. Most of our present knowledge of microbial physiology comes from research done in homogeneous batch cultures. The simplicity of the experiments made possible by this reductionist approach allowed for important findings, but it largely ignored the complexity of the microbial world. Extrapolating traits found in liquid to those that might be important in a community context could be deceptive. In the natural world, bacteria exhibit sophisticated multicellular behaviors and interact with one another, allowing them to carry out a wide range of activities that they could not otherwise carry out in liquid monocultures. A summary of a few key variables for the investigation of competition in biofilms can be found below [1]. The ability to live in groups or aggregates is a characteristic shared by all living things, including bacteria and mammals (Figure.1). The Allee effect, which is supported by a positive correlation between population size or density and means of individual fitness, postulates that there is an intrinsic advantage to grouping. This phenomenon has been widely studied; for example, it has been shown that grouping improves prokaryote resistance to desiccation and gives animals an improved defense against predators. It has also been shown that grouping affects the effectiveness of mating and reproduction in mobile organisms. *Streptococcus mutants*, which is a bacterium, are highly density-dependent when it comes to surviving under acidic stress while growing on the surface of teeth. Trade-offs between dispersal and survival in Escherichia coli populations and enhanced tolerance to several antibiotics when bacteria are at higher cellular densities are two additional instances of the Allee effect.

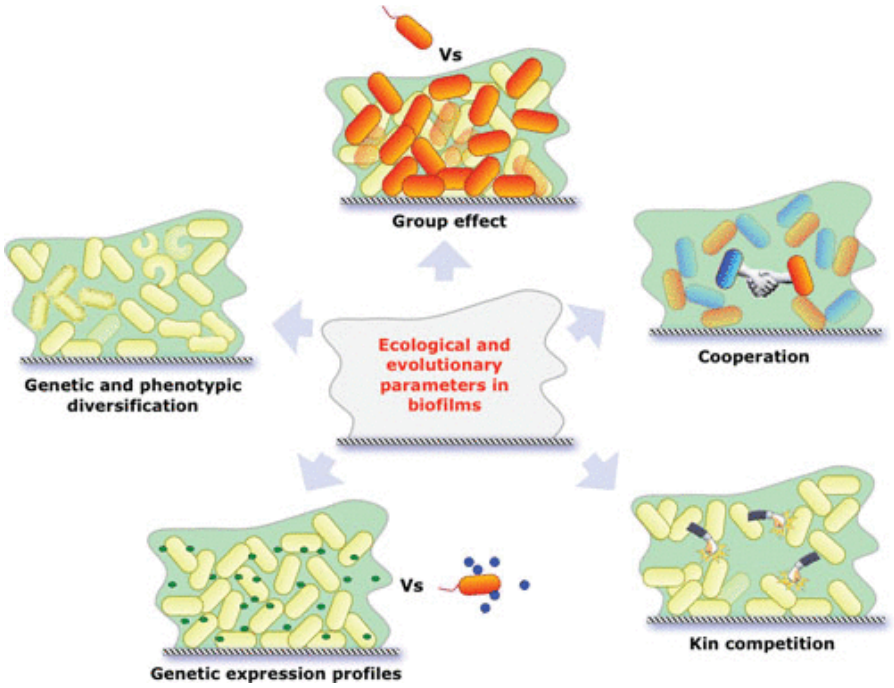

Figure 1: Parameter involve in the biofilms: Diagramed showing the different ecological and evolutionary parameters among the biofilms community(journal asm).

This antibiotic tolerance is unrelated to biofilm development, quorum sensing (QS), or antibiotic resistance mechanisms. Even though grouping is frequently advantageous, the physiological effects of higher cell density in bacterial clusters can make rivalry more intense. In the case of biofilms, bacteria are enclosed in a self-produced biofilm matrix that serves as a molecular sink or reservoir as a result of the matrix's restricted ability to retain substances and/or to allow for their outward diffusion. As a result, antagonist compounds produced by bacteria are more concentrated locally and are therefore more effective when fighting off nearby rivals. Last but not least, clustering strengthens competitive interactions, which are amplified when cellular densities are high and resources are scarce, for instance, through QS-dependent regulation and other mechanisms regulated by positive feedback loops [1].

Identifying bacterial kin requires three steps (Figure 2). First, receptor-ligand or receptor-receptor binding is how people first identify one another. Second, awareness is followed by a signal or biochemical action. The behavioral reaction comes in third. Studies in these species generally only require the observation of behavioral changes because brain cognition is typically involved in animal kin recognition. That is, kin recognition can be inferred from the way complete siblings are treated differently from nonkin. It can be challenging to research kin recognition in animals in part due to these challenges. In contrast, molecular events that can be seen immediately, like kin cells adhering to one another, are involved in bacterial kin recognition. A cooperative behavior that improves the fitness of the involved individuals is the outcome of these interactions [2].

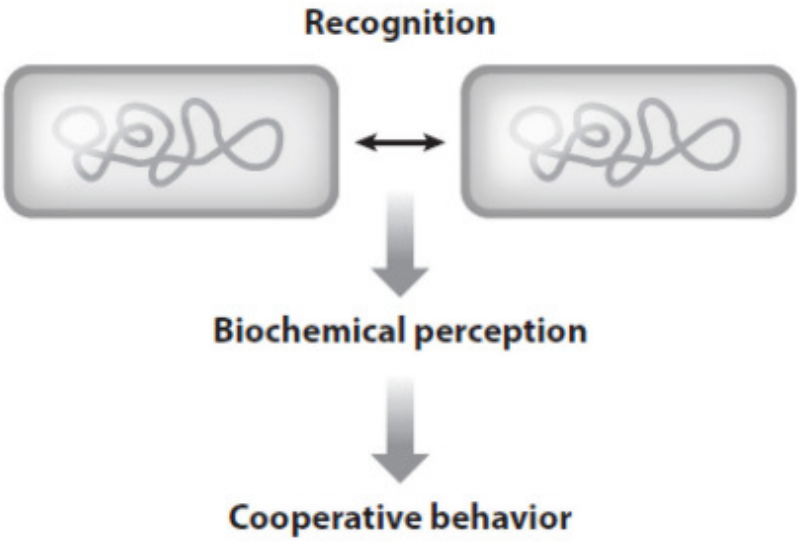

Figure 2: Kin recognition: Diagramed showing the kin recognition among the bacterial biofilms (NCBI).

Two categories can be used to classify ecological rivalry. The first kind, referred to as "exploitative competition," involves indirect interactions between organisms in which one organism restricts another organism's use of resources or stops them from accessing them altogether. Exploitative competition is widespread throughout the biological spectrum, but it is especially severe in biofilms because those habitats are already depleted of nutrients. Direct competition, also known as "interference competition," refers to specific mechanisms that harm rivals' chances of surviving or gaining access to a particular ecological niche or resource (Figure .3). The creation of antibiotics and other growth inhibitors that reduce bacterial viability serve as illustrations of this. Numerous interference tactics have recently been characterized in the context of biofilms that do not affect growth but do affect the

capacity to colonize a niche. Both exploitative and interfering competition processes have a significant impact on population dynamics and evolutionary outcomes. Theoretical studies suggest that the combination of these two strategies should lead to increased biodiversity or the coexistence of genotypes rather than exclusion or extinction [1] even though interactions between both mechanisms are still largely unexplored.

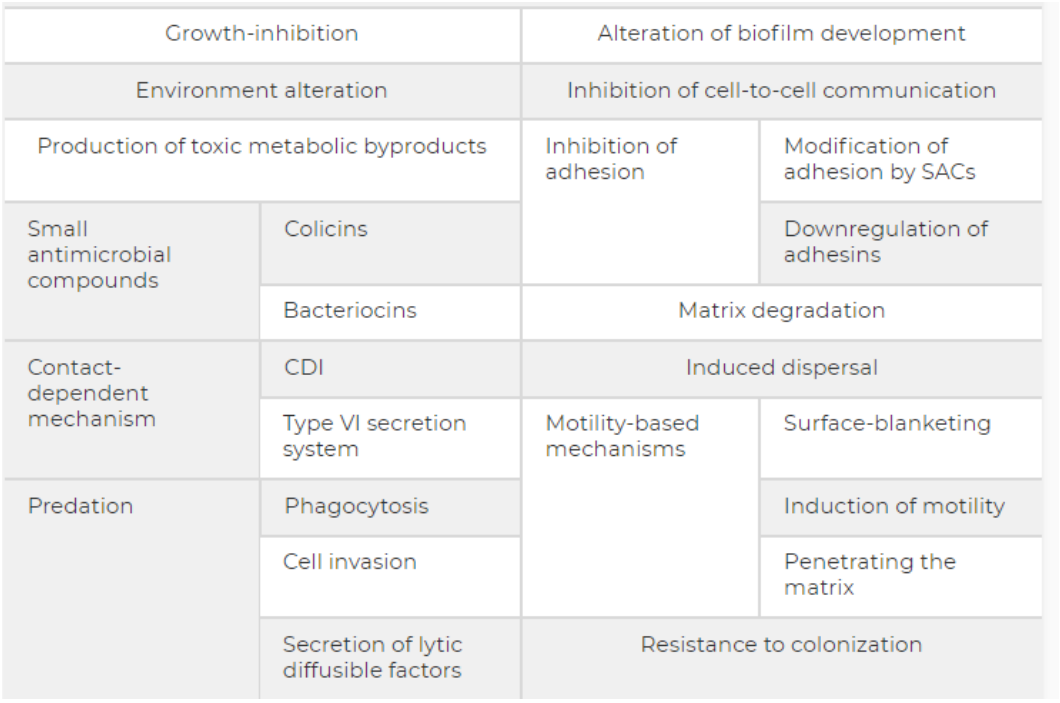

Figure 3: Interference competition: Diagram showing the example of the different Interference competitions among the biofilms (journal asm).

LITERATURE SURVEY

In patients with cystic fibrosis (CF), *Pseudomonas aeruginosa* is the most common source of morbidity and mortality. *P. aeruginosa* infections are challenging to treat because the organism is tending to create multicellular biofilms and various mechanisms of antibiotic resistance. *P. aeruginosa* epidemic strains frequently predominate in the lungs of distinct CF individuals, but it is unclear how they do this. *P. aeruginosa* strains can contend by producing pyocins, which are bacteriocins with chromosomal encoded instructions. In *P. aeruginosa*, soluble pyocins (S types) and tailocins are two of the three main classes of pyocin. (R and F types). We looked at the distribution of S- and R-type pyocins in 24 clinical strains isolated from distinct CF patients in this research before concentrating on their functions in interstrain competition. We found that (i) each strain produced only one R-pyocin type, but the number of S-pyocins varied between strains, (ii) R-pyocins were generally important for strain dominance during competition assays in planktonic cultures and biofilm communities in strains with both disparate R- and S-pyocin subtypes, and (iii) purified R-pyocins demonstrated significant antimicrobial activity against established biofilms. Our research supports a function for R-pyocins in the conflict between *P. aeruginosa* strains and sheds light on why some *P. aeruginosa* strains and lineages predominate over others during CF infection. Additionally, we show how R-pyocins have the potential to be used for therapeutic benefits at a time when antibiotic resistance is a major concern[3].

R-type tailocins are high-molecular-weight bacteriocins that are encoded in the genomes of numerous Pseudomonas species. They mimic bacteriophage tails. The *P. chlororaphis* 30-84

R-tailocin gene cluster was examined in this research, and it was found to contain the structural elements necessary to generate two R-tailocins with distinct ancestral origins. Transmission electron microscopy was used to identify two separate R-tailocin populations that had different lengths in the UV-induced lysates of P. chlororaphis 30-84. It was shown by mutants unable to produce either one or both R-tailocins that the lethal range of each tailocin is restricted to Pseudomonas species. Although a few Pseudomonas species were either killed by or resistant to both tailocins, the types of pseudomonads that the two R-tailocins killed varied. To show that the lysis cassette is necessary for the release of both R-tailocins, the holin gene within the tailocin gene cluster was deleted. The inability of P. chlororaphis 30-84 to contend with an R-tailocin-sensitive strain in biofilms and rhizosphere communities was caused by the loss of functional tailocin production. Our research shows that Pseudomonas species are capable of producing multiple effective R-tailocin ppapers that share the same lysis cassette but have different killing spectra. This research provides proof that R-tailocins play a crucial role in determining bacterial competition among Pseudomonas associated with plants in biofilms and the rhizosphere[4].

Communities of bacteria can communicate to plan their behavior. It has not been obvious whether these interactions can be used to guide the behavior of other populations that are far away. We found that electrical signaling can coordinate the growth dynamics of two Bacillus subtilis biofilm communities that are experiencing metabolic oscillations. Coupled with synchronized demand for scarce nutrients, coupling heightens rivalry. We confirm that biofilms resolve this conflict by switching from in-phase to antiphase oscillations, as anticipated by mathematical modeling. This leads to time-sharing behavior in which each group alternates between consuming food. Biofilms can develop counterintuitively faster when there is a lack of nutrients thanks to time-sharing. Through time-sharing, a technique used in engineered systems to distribute scarce resources, distant biofilms can thus coordinate their behavior to settle nutrient competition[5].

In microbial communities, the connection between biodiversity and ecosystem stability is not well known. Microbial electrolysis cells (MEC), which are small bioreactors, contain biofilm communities with a moderate number of species and easily tractable functional characteristics, making them the perfect setting for testing ecological hypotheses in microbial ecosystems. In this study, we examined the stability under a pH shock and the link between biodiversity and resilience in biofilm communities with a gradient of variety. The findings demonstrated that all bioreactors could resume steady performance following a pH disturbance, demonstrating a high degree of resilience. The resilient efficacy was demonstrated by the rebound of Geobacter and other exoelectrogens, and the presence of Methanobrevibacter may have slowed the functional recovery of biofilms, according to subsequent analysis of microbial composition. The microbial communities with greater variety tended to recover more quickly, suggesting that biofilms with greater biodiversity were more resilient to environmental disruption. Network analysis revealed that the negative interactions between the two dominant genera of Geobacter and Methanobrevibacter increased when the recovery time became longer, implying the internal resource or spatial competition of key functional taxa might fundamentally impact the resilience performances of biofilm communities. This research offers fresh perspectives on the connection between ecosystem functioning and diversity[6].

The types and quantities of nutrients present in the environment have an impact on the biofilms' final bacterial and chemical makeup as well as their growth. In oligotrophic environments, organisms react to nutrient stress by changing the morphology and surface characteristics of their cells, which improves adhesion. Little is known about how microbes

in animal oral cavities react to stress. In the oral cavity, the climate is less hostile, and nutrients are constantly available from saliva. Oral microbes work together metabolically to use the carbon and nitrogen in salivary glycoproteins. The environment in which oral bacteria develop can change how well those cells adhere to one another. Research on laboratory animals has demonstrated that feeding either glucose or sucrose diets or fasting has little impact on the early phases of oral biofilm development. Later on in the formation of the biofilm, however, diet may have an impact on the ratios of various bacterial species. The importance of carbon limitation and surplus as well as variations in environmental pH has been demonstrated by studies of population competition in oral bacterial communities in vitro and in vivo. The function of nitrogen metabolism in bacterial competition in biofilms has only received a small amount of research. Oral biofilms provide a sequestered habitat, similar to biofilms in nature, where organisms are shielded from removal by saliva and where cell interactions produce a biofilm ecosystem, different from that of saliva. Understanding the biology of oral biofilms has aided and will continue to aid in the prevention and treatment of these diseases[7]. Oral biofilms are an important component in the etiologies of caries and periodontal disease.

Because of the intricate interactions between microorganisms, substrate, operational conditions, and structure in micro bioreactors, simulating competition and development of numerous microorganisms is a well-known problem. In this study, we investigated the competitive biofilm formation of two aerobic nitrite and ammonium oxidizers in a microbioreactor using a multispecies thermal lattice Boltzmann model linked to a cellular automata model. To evaluate the effects of the structure and temperature on biofilm growth, detachment, and competition, three configurations of the microbioreactor with two heating blocks were simulated. The findings showed that the inlet port temperature had a greater impact on the biofilm development rate and pattern than did the temperatures and locations of the heating blocks. The biofilm development is more impacted by increasing the temperature of inlet 2 than by raising the temperatures of the two heating blocks. When the inlet temperature at inlet 2 rises from 10 to 50 °C, the percentage of the grids occupied by biofilm grows from 7.9 to 12.1%. Two microorganisms responded to changes in temperature and structure at two distinct rates. Compared to ammonium oxidizers, the nitrite oxidizer grew by about 20% more. We can comprehend how populations of biofilm and individual cells interact in the microbioreactor thanks to this model[8].

Although bacteria use intricate regulatory networks to deal with stress, little is known about how these networks operate in their natural environments. According to the competition sensing theory, bacterial stress response mechanisms can be used to identify ecological competition, but it can be difficult to study regulatory responses in different communities. Here, we use differential fluorescence induction to screen the Salmonella Typhimurium genome for loci that react, at the single-cell level, to life in biofilms with rival strains of S. Typhimurium and Escherichia coli. This solves the issue. According to this screening, the presence of competing strains increases the expression of genes linked to the production of biofilm matrix (CsgD pathway), epithelial invasion (SPI1 invasion system), and, ultimately, chemical efflux and antibiotic tolerance. (TolC efflux pump and AadA aminoglycoside 3-adenyltransferase). We confirm that the expected phenotypic changes in biofilm, mammalian cell invasion, and antibiotic tolerance are caused by these regulatory changes. We also demonstrate that these reactions result from the activation of major stress responses, directly supporting the idea of competition sense.

Further evidence that T6SS-derived cell damage activates these stress response systems is provided by the fact that inactivating a competitor's type VI secretion system (T6SS) stops

the responses to rivalry. Our research demonstrates that stress responses are used by bacteria to recognize and react to competition in a way that is crucial for the development of key phenotypes like biofilm formation, virulence, and antibiotic tolerance[9].

The microbial population structure and function of natural anaerobic communities maintained in laboratory fixed-bed biofilm reactors were tracked before and after a major perturbation, which involved the addition of sulfate to the influent of a reactor that had previously been fed only glucose (methanogenic), while sulfate was withheld from a reactor that had been fed both glucose and sulfate (sulfidogenic). The practical effectiveness of the biofilm reactors was connected to the population structure, which was established by using phylogenetically based oligonucleotide probes for methanogens and sulfate-reducing bacteria. Even though sulfate was not present in the reactor's influent, the methanogenic reactor had up to 25% methanogens and 15% sulfate-reducing microbes before the perturbation. The most prevalent methanogens and sulfate-reducing bacteria, respectively, were Methanobacteriales and Desulfovibrio species. Due to their capacity to operate as proton-reducing acetogens and/or fermenters, sulfate-reducing bacteria (primarily Desulfovibrio spp. and Desulfobacterium spp.) can exist in the absence of sulfate. Sulfate reduction began immediately following the addition of sulfate consistent with the presence of significant levels of sulfate-reducing bacteria in the methanogenic reactor, and levels of sulfate-reducing bacteria increased to a new steady-state level of 30 to 40%; coincidentally, effluent acetate concentrations decreased. Notably, the Desulfococcus/Desulfosarcina/Desulfobotulus group of sulfate-reducing bacteria was more effective without sulfate. Following the addition of sulfate, methane production immediately dropped; this was then followed by a decrease in the relative concentration of methanogens, which eventually reached a new steady-state level of about 8%. The sulfidogenic reactor's transition to a sulfate-free medium did not quickly switch to methanogenesis. Only about 50 days after the disturbance was methane production and a significant rise in the number of methanogens noted[10].

CONCLUSION

The formation of bacterial biofilms, which are dense surface-associated communities, is essential to the persistence of bacteria and how they impact us. Most people think of biofilm formation as a collaborative process where different strains and species work together to achieve a shared objective. Here, we investigate a different hypothesis: biofilm development is a reaction to ecological competition. It is also demonstrated that pyocins, a class of narrow-spectrum antibiotics produced by other *P. aeruginosa* strains, can promote biofilm development by enhancing cell attachment. Side-by-side comparisons using microfluidic assays suggest that the increase in biofilm occurs due to a general response to cellular damage: a comparable biofilm response occurs for pyocins that disrupt membranes as for commercial antibiotics that damage DNA, and inhibit protein synthesis or transcription. Our findings demonstrate that in reaction to ecological competition, which is picked up by antibiotic stress, bacteria increase biofilm formation. This contradicts the frequently reached conclusion that cooperative signals that organize microbial communities exist at sub-lethal antibiotic concentrations. Research findings support the use of low doses of antibiotics in competition sensing, which allows for the detection and reaction to competing genotypes that generate them. Bacteria frequently form biofilms by adhering to one another and surfaces. These dense communities are present everywhere and play a key role in both health and illness, including on and inside of us. Many times, the formation of biofilms is regarded as the coordinated action of various strains that cooperate to thrive and defend themselves. In research, it was supported by a strikingly different perspective: When bacterial strains fight with one another, biofilms are created.

REFERENCES:

[1] O. Rendueles and J.-M. Ghigo, "Mechanisms of Competition in Biofilm Communities," *Microbiol. Spectr.*, 2015, doi: 10.1128/microbiolspec.mb-0009-2014.

[2] D. Wall, "Kin Recognition in Bacteria," *Annual Review of Microbiology*. 2016. doi: 10.1146/annurev-micro-102215-095325.

[3] O. Oluyombo, C. N. Penfold, and S. P. Diggle, "Competition in biofilms between cystic fibrosis isolates of pseudomonas aeruginosa is shaped by R-pyocins," *MBio*, 2019, doi: 10.1128/mBio.01828-18.

[4] R. J. Dorosky, J. M. Yu, L. S. Pierson, and E. A. Pierson, "Pseudomonas chlororaphis produces two distinct R-tailocins that contribute to bacterial competition in biofilms and on roots," *Appl. Environ. Microbiol.*, 2017, doi: 10.1128/AEM.00706-17.

[5] J. Liu *et al.*, "Coupling between distant biofilms and emergence of nutrient time-sharing," *Science (80-.).*, 2017, doi: 10.1126/science.aah4204.

[6] K. Feng *et al.*, "Biodiversity and species competition regulate the resilience of microbial biofilm community," *Mol. Ecol.*, 2017, doi: 10.1111/mec.14356.

[7] G. H. Bowden and Y. H. Li, "Nutritional influences on biofilm development.," *Advances in dental research*. 1997. doi: 10.1177/08959374970110012101.

[8] M. Aghajani Delavar and J. Wang, "Modeling Combined Effects of Temperature and Structure on Competition and Growth of Multispecies Biofilms in Microbioreactors," *Ind. Eng. Chem. Res.*, 2020, doi: 10.1021/acs.iecr.0c03102.

[9] B. Lories, S. Roberfroid, L. Dieltjens, D. De Coster, K. R. Foster, and H. P. Steenackers, "Biofilm Bacteria Use Stress Responses to Detect and Respond to Competitors," *Curr. Biol.*, 2020, doi: 10.1016/j.cub.2020.01.065.

[10] L. Raskin, B. E. Rittmann, and D. A. Stahl, "Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms," *Appl. Environ. Microbiol.*, 1996, doi: 10.1128/aem.62.10.3847-3857.1996.

CHAPTER 21

BIOFILM'S SIGNIFICANT ROLE IN THE OIL AND GAS INDUSTRY

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ABSTRACT:

All over the globe, there are microorganisms in oil reservoirs that cause oil to deteriorate and change in quality. Due to the scarcity of undisturbed samples, our understanding of the processes in deep oil reservoirs is sadly restricted. The distribution of microorganisms in the oil-water transition zone and the oil leg's water-saturated regions, as well as any potential physiological adjustments to biotic and abiotic ecological variables like temperature, salinity, and viruses, are covered in this review. Because tiny water inclusions and pockets within the oil leg offer an exceptional habitat for microorganisms within a natural oil reservoir and simultaneously widen the zone of oil biodegradation, we demonstrate the significance of investigating the water phase within the oil. Oil biodegradation is influenced by environmental variables such as temperature and salinity. The kind of microbes that can live in the reservoir depends on temperature. Proteobacteria and Euryarchaeota are present in hydrocarbon reservoirs at all temperatures, while other organisms are only present at certain temperatures. The main mode of life in oil reserves is hypothesized to be biofilm formation, which improves nutrient uptake, syntrophic interactions, and environmental stress resistance. The abundance of viruses in oil reservoirs has been documented in the literature, and it has been debated how this may affect the makeup of the microbial community.

KEYWORDS:

Oil Reservoirs, Gas Reservoirs, Gas Industry, Microbial Community, Microbiologically Environment.

INTRODUCTION

Microbial processes are crucial for the industry, environmental security, and many other aspects of the economy. The Microbiology Department of the Oil and Gas Institute has carried out research using various microbiological techniques and technologies. Interest in these processes and their practical application to prospecting for and exploring hydrocarbon deposits have grown quickly in recent years. The oil and gas business has already adopted the findings of this research, which have practical applications. The oil and gas industry continues to find microbiological techniques appealing, so the methodology is being improved and changed to address current needs and issues. The rational prospecting for and investigation of hydrocarbon deposits can make use of microorganisms and biogenic processes. However, unchecked and excessive microbial growth can result in bacterial contamination, such as the biodegradation of drilling fluids, microbiologically influenced corrosion, and microbial contamination of oil and gas that has been kept.

These bacteria are found to be particularly enriched in the near-surface soils and sediments above the oil and gas reservoirs and rely solely on hydrocarbon gases as sustenance. The potential for hydrocarbon extraction can be assessed by finding abnormal populations of bacteria that oxidize n-pentane and n-hexane in the surface soils. The Bikaner Nagaur basin has undergone a geo-microbial survey to look into the potential for hydrocarbon development. In the current research, n-pentane-using bacteria have bacterial counts between

2.0 102 and 1.26 106 cfu/gm, while n-hexane-using bacteria have bacterial counts between 2.0 102 and 1.21 106 cfu/gm. Four different anomalies can be seen in the study area according to the distribution maps of bacterial concentration. The hydrocarbon-oxidizing bacteria range between 103 and 106 cfu/gm in soil/sediment receiving hydrocarbon micro-seepages, emphasizing the potential of finding oil or gas reservoirs using the microbiological method. Between 105 and 106 cfu/gm of soil sample are discovered to be n-pentane and n-hexane-using bacteria in the Bikaner Nagaur basin, which is significant and supports the seepage of lighter hydrocarbon accumulations from oil and gas reservoirs. According to geo-microbial prospecting studies, the study region contains hydrocarbon micro-seepage with the subsurface origin, which suggests that there are good chances for finding oil there [1].

Despite having a high level of microbial resilience, the biological decomposition of many natural, semi-synthetic, and synthetic polymers is well documented in the literature. By breaking down the bonds in polymer molecules, particular bacterial enzymes can disclose the monomeric units that microorganisms use as sources of carbon, nitrogen, and phosphorus (Figure.1). Polymeric compounds have been used in drilling fluid technology for a very long time. Drilling fluids based on organic polymers are safer, less toxic, and more ecologically friendly than conventional fluids. However, due to their high susceptibility to biodegradation, polymers agents in particular must be protected against it by using the proper biocidal agents. The cleanup of used drilling fluids, however, benefits from the biodegradation process [2].

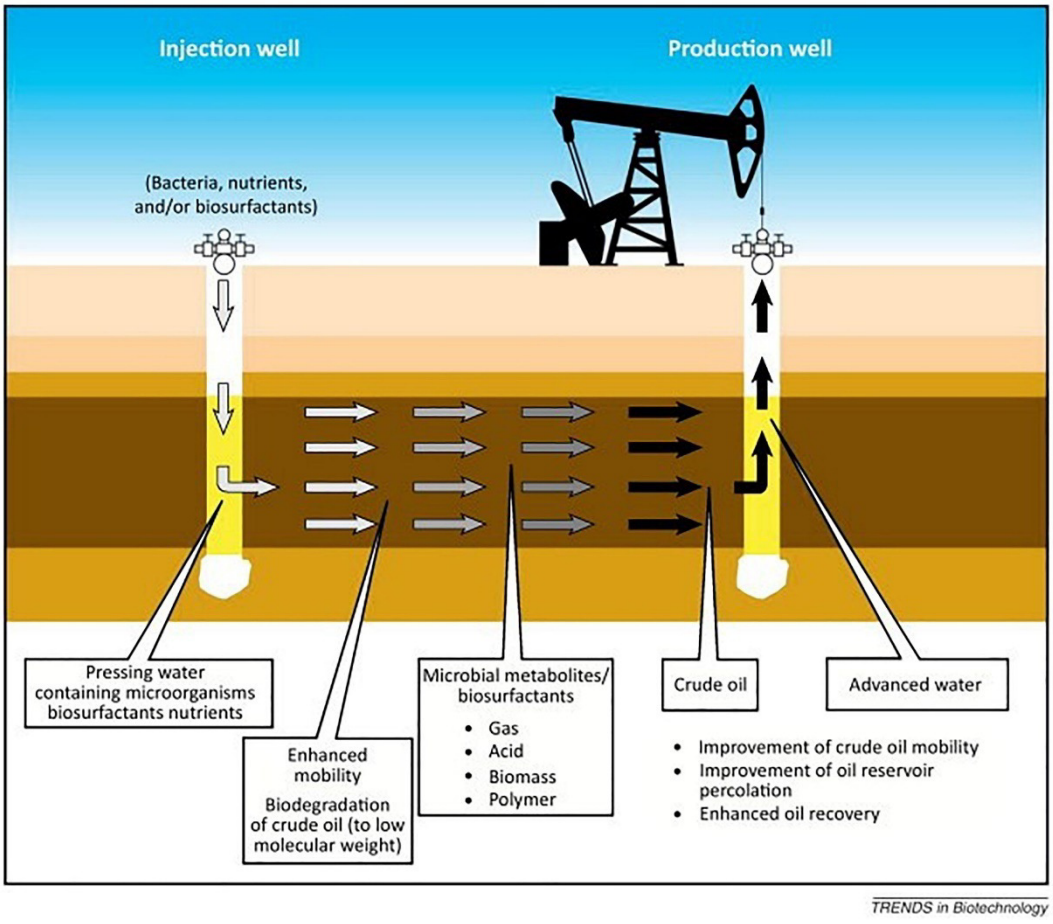

Figure 1: Role of microorganism: Diagramed showing the different roles of the microorganism in the different industries (frontier).

A biologically based technique known as "microbial enhanced oil recovery" (MEOR) involves controlling the structure, function, or both of the microbial environments found in

oil reservoirs. The final goal of MEOR is to enhance oil recovery from porous media while boosting economic output. The tertiary oil extraction method known as MEOR allows for the partial recovery of the two-thirds of oil that are typically left over, prolonging the life of mature oil reservoirs.

MEOR is a multidisciplinary subject that includes, among other things, chemical engineering, fluid mechanics, petroleum engineering, chemistry, microbiology, and geology. The microbial processes proceeding in MEOR can be classified according to the oil production problem in the field: wellbore clean-up removes mud and other debris blocking the channels where oil flows through; well stimulation improves the flow of oil from the drainage area into the well bore; and enhanced water floods through stimulating microbial activity by injecting selected nutrients and sometimes indigenous microbes. MEOR is a system made up of a reservoir, microbes, nutrients, and a well injection procedure from an engineering perspective. Increase oil recovery from multistage fractured horizontal shale oil wells that are running out of oil in nontraditional shale oil reservoirs.

Controlling the bacterial and chemical condition of the water in a hydrocarbon deposit, as well as the composition of natural gas in storage, is required to effectively avoid the processes that result in the formation of H2S in deposits and microbiologically influenced corrosion. Because underground gas storage regulates Poland's daily, monthly, and seasonal gas needs and ensures its energy security, microbiologically induced corrosion there is a particularly risky occurrence. The tasks of separating sulfate-reducing and sulfate-oxidizing bacteria from the deposits and choosing effective antibacterial substances were completed, carried out at various storage sites, and led to many practical applications.

The total removal of hydrocarbon contaminants is significantly aided by naturally existing microorganisms found in soil matrices. Significant portions of aerobic remediation of surface pollutants are contributed by bacterial and fungal degradation processes. This research examined how naturally occurring microbial communities in laboratory microcosms under optimum environmental conditions degraded conventional diesel, heating diesel fuel, synthetic diesel (Syntroleum), fish biodiesel, and a 20% biodiesel/diesel blend. The Syntroleum and fish biodiesel contaminated samples, which also displayed the greatest total hydrocarbon mineralization (>48%) during the first 28 days of the experiment, exhibited visible microbial remediation. The lowest total hydrocarbon mineralization was observed during the heating of diesel and conventional diesel fuels, with 18–23% under optimum circumstances. Fungi were able to live and grow using only volatile hydrocarbon compounds as a carbon source in concurrent tests with the growth of fungi suspended on a grid in the air space above a particular fuel with little to no soil. For all five of the studied fuel types, these configurations involved minimal bacterial degradation. The families Giberella, Mortierella, Fusarium, Trichoderma, and Penicillium are home to fungi that can flourish on particular hydrocarbon substrates [3].

LITERATURE SURVEY

Even though the development of bacterial biofilms is directly related to microbially influenced corrosion (MIC) and reservoir souring, sessile bacteria that create these biofilms are still not always monitored. This paper discusses the necessity of tracking sessile bacteria in biofilms as well as the variety of tracking methods accessible, from direct microscopic inspection to genetic methods. The paper emphasizes the critical necessity of biofilm monitoring to effectively apply biocides, nitrate, etc. to control their problematic activity. The paper shows how, when used properly, the presently available bacterial monitoring methodology can produce valuable, representative, and repeatable data for tracking bacterial

contamination and maximizing the effectiveness of control measures in oilfield samples. The message must be accepted by those responsible for bacterial monitoring and control that until they begin to rigorously apply the standard methods already available to them there is little hope of any progress in the overall control of problematic bacterial activity in the oilfield for the foreseeable future [4].

Biofilms are an ingenious form of life that bacteria and other microorganisms have developed where they cooperate and increase their possibilities of survival when faced with environmental stress. A matrix of extracellular polymeric substances that protects bacteria from factors like temperature and pH changes, UV exposure, changes in salinity, nutrient loss, antimicrobial chemicals, and predators is present in these communities of adhered cells. They are able to colonize almost all man-made surfaces in touch with seawater due to their success in marine environments and the abundance of bacterial cells in the sea. The significant costs to maritime transportation, aquaculture, oil and gas industries, desalination plants, and other industries have prompted the creation of several strategies to stop the formation of biofilms and clean infected surfaces. The advantages of bacterial cells living in biofilms and their effects on human actions are covered in this review [5].

While the oil and gas industry has witnessed increased applications of molecular microbiological methods (MMMs) for diagnosing and managing microbiologically influenced corrosion (MIC) in the past decade, the process of establishing clear links between microbiological conditions and corrosion mechanisms is still emerging. Different MMMs offer different kinds of information about microbial diversity, abundance, activity, and function, all of which are very dissimilar from the findings obtained using cultures that are known to corrosion specialists in the oil and gas industry. Additionally, a multidisciplinary method for determining the importance of molecular microbiological data concerning identifying, mitigating, and monitoring corrosion threats has not yet been clearly defined. The advantages of using MMMs for MIC management are thus not yet completely understood or appreciated. Despite technological advancements, many oil and gas asset managers won't accept the microbiological insights provided by MMMs until their importance concerning corrosion management and asset integrity is made clearer. This paper [6] discusses the necessity of a project that brings disciplinary experts, microbiological technologies, and corrosion experts together to achieve a shared understanding.

During oil and gas operations, pipeline networks and related infrastructures are exposed to various corrosion deterioration mechanisms, one of which is microbiologically influenced corrosion (MIC). MIC results from accelerated disintegration initiated by various microbial exercises present in oil and gas frameworks. A biofilm, which includes numerous bacterial cells, extracellular polymeric substances (EPS), and rust products, develops when microorganisms are present. In-depth explanations of the various microorganisms engaged in MIC have been provided. Sulfate-reducing bacteria (SRB) are frequently found in oilfields and are the primary source of souring. A concise description of the physical, chemical, electrochemical, and biological alleviation strategies for MIC was explored and discussed. The importance of MIC and various protection strategies were also introduced. Biological treatments, or using various types of bacteria against the main bacteria that cause MIC in a specific industrial situation, are the most recent technique for MIC mitigation. Finally, future views are discussed, which will aid researchers in coming up with fresh MIC mitigation strategies[7]. Carbon steel in oil and gas pipeline networks has long been thought to deteriorate due to microbially influenced corrosion (MIC). To find and describe sessile biofilm communities in a high-temperature oil production pipeline and to compare the profiles of the biofilm community with those of the previously studied planktonic

communities, the authors set out to identify and characterize these communities. Mesophilic and thermophilic sulfidogenic anaerobes, as well as eubacterial and archaeal 16S rRNA sequences of DNA, recovered from extracted pipeline fragments, or "cookies," were found there. Sessile cells and chemical components typical of corrosive biofilms were found in the cookies after elemental analysis and electron imaging was performed. Putative hydrocarbon metabolites were discovered by mass spectrometry in cookie acid washes, and surface analysis showed pitting and general corrosion damage. The findings imply that the planktonic eubacterial and archaeal communities are represented by the biofilm taxa in an established closed system, and that sampling and tracking of the planktonic bacterial population can provide information about biocorrosion activity. Additionally, these groups are probably able to survive thanks to hydrocarbon biodegradation. The significance of proper sample handling and storage methods is emphasized for oilfield MIC diagnostics[8].

By combining microsensor profiling, 15N, and 35S labeling, and 16S rRNA gene-based fingerprinting, we investigated the effects of NO3 on the composition, diversity, and function of the bacterial population in situ industrial, anaerobic biofilms. In a device created to clean seawater for injection into an oil field for pressurized hydrocarbon recovery, biofilms were grown on carbon steel coupons. To stop bacterial H2S production and microbially influenced corrosion in the field, NO3 was introduced to the seawater. The zone of greatest metabolic activity within the biofilms was found to be near the metal surface, correlating with a high bacterial abundance in this region, according to the micro-profiling of nitrogen compounds and redox potential inside the biofilms. The majority of NO3 was converted to NO2 upon addition. Redox potentials of 450 mV at the metal surface in biofilms developed without NO3 indicated the release of Fe2+. With the largest number of obtained clones in the clone library belonging to sequences related to Methylophaga and Colwellia, NO3 addition to previously untreated biofilms resulted in a decline (65%) in bacterial species richness. The community composition did not alter, however, and there was no potential reduction in NO3 after the later withdrawal of NO3. All biofilms had active sulfate reduction below detection levels, but sulfide deposits' S isotope fractionation studies indicated that it must have happened either slowly or sporadically. According to scanning electron microscopy, pitting erosion happened on all coupons, regardless of the treatment. However, the addition of NO3 reduced uniform corrosion[9].

Microbial communities that can take part in harmful processes like biocorrosion are abundant and varied in the oil-water-gas environments of oil production sites. The microbial communities from an oil extraction facility on the Alaskan North Slope were characterized using several molecular techniques, such as pyrosequencing of 16S rRNA libraries. In order to pinpoint particular populations or communities linked to biocorrosion, the communities in produced water and a sample from a "pig envelope" were compared. The samples are richer in surface-associated solids (such as paraffin, minerals, and biofilm), which are used to physically mitigate pipeline corrosion and fouling. Coincidentally, microorganisms are also present in the samples (over 105-fold). Bacteria were 10–150 times more prevalent than archaea throughout the oil production plant, and the thermophilic members of the phyla Firmicutes (Thermoanaerobacter and Thermacetogenium) and Synergistes (Thermovirga) dominated the community. Thermacetogenium and Thermovirga had higher relative abundances, which gave the microbial community in the pig envelope a unique structure (relative abundances of taxa). The information provided here suggests that biofilm communities linked to biocorrosion are best represented by bulk fluid, but that some populations are more prevalent in biofilms and should be the focus of methods for monitoring and mitigation [10].

CONCLUSION

The finding of petroleum hydrocarbons, bacterial enhanced oil recovery (BEOR), solubilization, emulsification, and bioremediation of petroleum are all examples of the roles and uses of bacteria in the petroleum industry. Several organisms of microbes have developed that can oxidize oil and its materials for their only origin of carbon dioxide and power, similar bacteria had performed an essential function in the procedure of oil creation by triggering a lot of natural responses in the underwater ppapers rich in natural significance. It was determined that the most effective and economical method for improving oil recovery from mature reservoirs or abandoned wells was bacterial-enhanced oil recovery technology. However, bacteria created biological surface-active compounds (biosurfactants), which can decrease surface tension and interfacial tension in petroleum mixtures. In recent years, chemically synthesized surface-active agents were used to improve oil recovery (EOR). This chapter summarized the overview of the advantage of microorganisms involved in the oil and gas industry.

REFERENCES:

[1] M. A. Rasheed, M. Lakshmi, D. Srinu, and A. M. Dayal, "Bacteria as indicators for finding oil and gas reservoirs: A case study of the Bikaner-Nagaur Basin, Rajasthan, India," *Pet. Sci.*, vol. 8, no. 3, pp. 264–268, 2011, doi: 10.1007/s12182-011-0143-z.

[2] A. Turkiewicz, "The role of microorganisms in the oil and gas industry," *Rocz. Ochr. Sr.*, 2011.

[3] A. Horel and S. Schiewer, "Microbial degradation of different hydrocarbon fuels with mycoremediation of volatiles," *Microorganisms*, 2020, doi: 10.3390/microorganisms8020163.

[4] S. Maxwell, C. Devine, F. Rooney, and I. Spark, "Monitoring and control of bacterial biofilms in oilfield water handling systems," in *NACE Meeting Papers*, 2004.

[5] C. C. C. R. de Carvalho, "Marine biofilms: A successful microbial strategy with economic implications," *Frontiers in Marine Science*. 2018. doi: 10.3389/fmars.2018.00126.

[6] R. B. Eckert and T. L. Skovhus, "Advances in the application of molecular microbiological methods in the oil and gas industry and links to microbiologically influenced corrosion," *Int. Biodeterior. Biodegrad.*, 2018, doi: 10.1016/j.ibiod.2016.11.019.

[7] B. Chugh, S. Thakur, and A. K. Singh, "Microbiologically influenced corrosion inhibition in oil and gas industry," in *Corrosion Inhibitors in the Oil and Gas Industry*, 2020. doi: 10.1002/9783527822140.ch13.

[8] T. R. Lenhart *et al.*, "Identification and characterization of microbial biofilm communities associated with corroded oil pipeline surfaces," *Biofouling*, 2014, doi: 10.1080/08927014.2014.931379.

[9] C. U. Schwermer *et al.*, "Impact of nitrate on the structure and function of bacterial biofilm communities in pipelines used for injection of seawater into oil fields," *Appl. Environ. Microbiol.*, 2008, doi: 10.1128/AEM.02027-07.

[10] B. S. Stevenson, H. S. Drilling, P. A. Lawson, K. E. Duncan, V. A. Parisi, and J. M. Suflita, "Microbial communities in bulk fluids and biofilms of an oil facility have similar composition but different structure," *Environ. Microbiol.*, 2011, doi: 10.1111/j.1462-2920.2010.02413.x.

CHAPTER 22

DIFFERENT CONDITIONS INVOLVED IN THE BIOFILM FORMATION IN STAPHYLOCOCCUS AUREUS

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ABSTRACT:

Due to their difficult and ineffective treatment by antibiotics, staphylococcal infections are known to cause very serious problems in hospitalized and immunocompromised patients globally. Efflux pumps, gene products whose expression is altered by quorum sensing, and bacteria embedded in biofilms that become resistant to the immune system and antibiotics are the main culprits behind chronic and recurring infections like infections linked to indwelling medical devices. Biofilm-embedded sessile communities have diverse cell populations with a variety of antimicrobial responses. *Staphylococcus epidermidis* (*S. epidermidis*) and Staphylococcus aureus (*S. aureus*) are primarily known infectious strains can create gene expression of biofilm that has an essential role in the cause of staphylococcal diseases and leads to bacterial connection and immigration on biological such as cells or abiotic areas such as artificial surfaces that may act as a substrate for bacteria adhesion when bacteria subjected for stress limitations. The entire body becomes infected as a result of the bacteria in this expressed and developed biofilm spreading throughout the body. Biofilm infections are difficult to treat, and novel agents are being studied to stop the growth and spread of biofilm. Establishing the infectiousness and the function of biofilm of *S. epidermidis* and S. aureus in persistent diseases such as implanted device–associated infections, the process and the worldwide control of biofilm manufacture by the quorum-sensing system, inactivation of biofilm formation, and the opposition structures of biofilm-embedded bacteria toward antimicrobial agents is important.

KEYWORDS:

Adhesion Pia, Biofilm Infection, Bacterial Biofilms, Bacterial Infection, Staphylococcus Aureus

INTRODUCTION

One of the most prevalent bacterial pathogens that colonize the epidermis and/or mucosal membranes of mammals is *Staphylococcus aureus*. As a commensal bacteria and an opportunistic pathogen that causes a broad range of infections, including simple soft tissue infections, endocarditis, bacteremia, and severe pneumonia, *S. aureus* has significant clinical significance. Biofilms are highly organized multicellular bacterial communities that are encased in a complex matrix made of proteins, polysaccharides, and/or extracellular DNA (eDNA). Biofilms increase the persistence of biofilm-associated infections and decrease their susceptibility to antimicrobials. Bacterial biofilm formation is an important part of how they survive in the host and is thought to be a major contributor to their virulence, which is what causes severe chronic infections. The ability to form biofilms is important for *S. aureus* pathogenicity in clinical settings, such as indwelling medical devices or catheter-associated infections, and biofilm-associated S. aureus infections resist antimicrobial therapy and innate host defense mechanisms. Furthermore, the development of biofilms by *S. aureus* and antimicrobial resistance are physiologically related because the expression of the biofilm

phenotype can be affected by the development of antimicrobial resistance. Biofilms are linked to several infectious illnesses, which are now being recognized as an urgent public health issues. The *S. aureus* that causes biofilm-associated infections may come from various genetic backgrounds and, as a result, may exhibit a variety of virulence factors when infected. For instance, it seems that the agr quorum sensing system is connected to several regulatory variables, including the formation of biofilms. Agr increases the expression of several toxins, such as -toxin, a molecule with surfactant-like characteristics that aid in S. aureus adhesion and the formation of biofilms. Additionally, polysaccharide intercellular adhesin (PIA), which is generated and controlled by the intercellular adhesion (ica) ADCB operon, is necessary for biofilm development. An N-acetylglucosamine transferase (ICAA and ICAB), a deacetylase (ICAD), and a projected exporter are all present in the icaADCB operon. (icaC). Additionally, some surface elements, like Staphylococcal protein A (spa), aid in the adherence of biofilms. Genotypic differences between S. aureus strains may also affect biofilm development, but these correlations are not always noted. Additionally, the development of biofilms is related to the genetic epidemiology of methicillin-resistant S. aureus (MRSA) strains, as determined by staphylococcal cassette chromosome mec (SCCmec) typing[1].

A biofilm is described as a sessile microbial community in which cells are embedded in a protective extracellular polymeric matrix and attached to a surface or other cells. Gene expression and protein production are altered physiologies during this mode of development. There are at least three main events that can be used to categorize the various biofilm developmental stages: initial attachment, biofilm maturation, and dispersal. (Figure 1A). An individual planktonic cell initially attaches to a surface by reversibly associating with it; if the cell does not dissociate, it binds permanently to the surface. Surface proteins, also known as microbial surface components that recognize sticky matrix molecules, play a role in attachment. (MSCRAMMs). These proteins have a significant impact on host factors like fibrinogen, fibronectin, and collagen during infection. Cell proliferation and the creation of the extracellular polymeric matrix are two processes that lead to biofilm maturation. Although the biofilm matrix can contain host factors, polysaccharides, proteins, and extracellular DNA, the composition differs between strains. (eDNA). Cells within the biofilm can reactivate to a planktonic form through dispersal after biofilm accumulation. In this review, the main S. aureus dispersal processes will be examined.

Biofilms not only provide resistance to clearance processes but also significantly contribute to the development of chronic diseases. After a biofilm has been established, individual cells may separate from it and either spread the infection to new areas or cause a severe infection like sepsis. This hypothesis is supported by the function of the S. aureus quorum sensing system during dispersal. Due to its significance in chronic infections and the biofilm model of growth, dispersal has been the subject of many recent studies. An analysis of key dispersal mechanisms has resulted in the creation of dispersal-mediated treatment options for biofilm infections. The main processes for *S. aureus* biofilm dispersal are covered in this review. The model of *Staphylococcus aureus* biofilm development analyzes the possibility of creating dispersal-mediated therapies for biofilm infections (Figure 1B)[2].

The five phases of S. aureus biofilm formation are attachment, multiplication, exodus, maturation, and dispersal (A–E). Using hydrophobic contacts or MSCRAMMs, A. S. aureus cells cling to biotic or abiotic surfaces, respectively. B. Following cell attachment, the biofilm transforms into a confluent "mat" of cells made up of a proteinaceous framework and eDNA. C. After confluency, there is a time of mass cell exodus during which a portion of the cell population is liberated from the biofilm by Sae-regulated nuclease-mediated eDNA

degradation, enabling the development of three-dimensional microcolonies. D. Specific clusters of cells that stayed connected during the exodus stage develop into microcolonies. Rapid cell division during this period creates strong aggregations of proteins, including PSMs and eDNA. E. Via the stimulation of proteases and/or the production of PSM, activated Agr-mediated quorum sensing starts the modification of the biofilm matrix and the dispersion of cells. Autolysin A, MSCRAMM (microbial surface components recognizing adhesive matrix molecules), PSM (phenol soluble modules), eDNA (extracellular DNA), and Agr (accessory gene regulator) are some of the terms used.

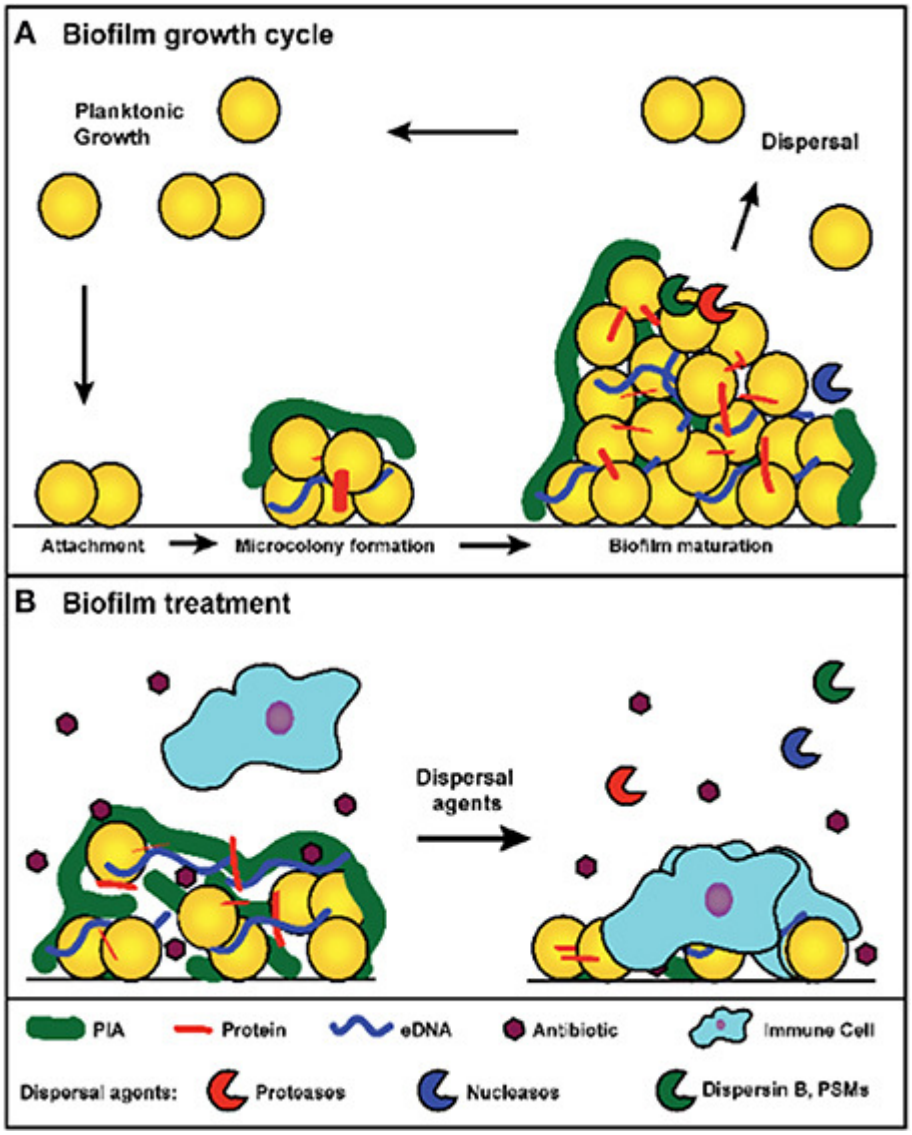

Figure 1: Stages of the *Staphylococcus aureus* developments: Diagramed showing the different stages of the Staphylococcus aureus development (Frontier).

Numerous extracellular proteins made by staphylococci may help biofilm buildup by encouraging intercellular binding soon after initial attachment. Some of these MSCRAMM-designated CWA proteins, such as the FnBPs, ClfB, and SdrC proteins, have dual functions in both attachment and aggregation. Other CWA proteins, including the S. aureus homolog SasG and the *Staphylococcus epidermidis* accumulation-associated protein (Aap), have also been linked to adhesion and early accumulation. Additionally, CWA proteins like Protein A, SasC, and Bap have all demonstrated a tendency to promote the development of biofilms. Although these proteins seem to play a part in the multiplication stage of biofilm formation,

flow-cell experiments lacking matrix components did not reveal their function during this stage. Similar to this, it has been demonstrated that polysaccharide intracellular adhesin (PIA) contributes to the early *S. aureus* biofilm development by acting as an ECM component; however, the production of this matrix molecule seems to be strain- or condition-dependent. Indeed, descendants of the UAMS-1 and USA300 JE2 strains that were icaA mutants (a gene encoding an N-glycosyltransferase required for PIA production) showed typical accumulation during the multiplication stage (Figure.2) [3]. In summary, this chapter covers the five stages of *S. aureus* biofilm development: attachment, multiplication, exodus, maturation, and dispersal. We also address the molecular mechanisms involved in each step.

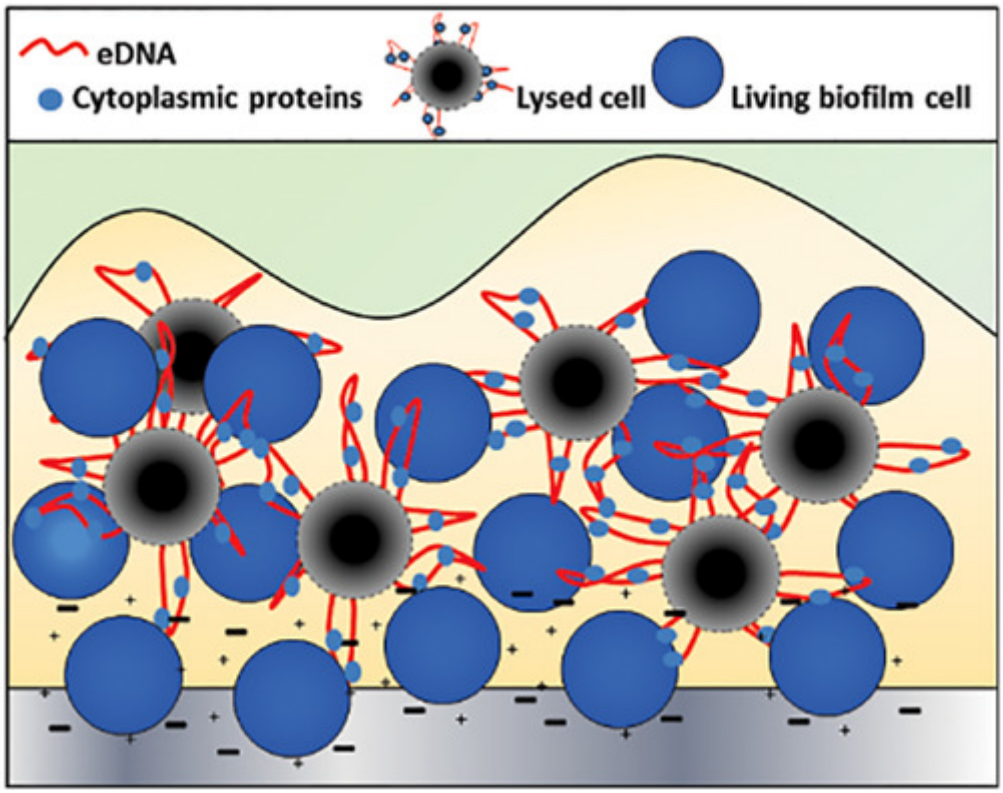

Figure 2: *Staphylococcus aureus* multiplication: Diagram showing the Staphylococcus aureus multiplication stage (online library).

LITERATURE SURVEY

Staphylococcus aureus and the development of its biofilm are acknowledged as significant clinical issues. S. aureus is a food-borne pathogen as well, but little is known about how strains associated with food create biofilms. We have investigated the development of biofilms in strains of S. aureus that are used in food preparation as well as clinical strains that are grown under various stress conditions, including temperature, sodium chloride, glucose, and ethanol. S. aureus strains associated with food were found to be strong biofilm formers, and environmental factors pertinent to the food business had an impact on biofilm formation. The findings demonstrated that biofilm production was enhanced at less-than-ideal temperatures for growth. Glucose and sodium chloride together facilitated the development of the biofilm. The expression of several biofilm-related genes was influenced by both temperature and osmolarity. (e.g. icaA and rbf). Additionally, differences in gene expression (such as icaA, agrA, and sigB) between genotypes were noted. Our findings confirm the presence of *S. aureus* biofilm production mechanisms that are both ica-dependent and ica-independent. The findings of the phenotypic and genotypic analyses revealed extremely

varied and intricate patterns of biofilm formation in S. aureus. This exemplifies the need for caution when making generalizations about S. aureus gene expression in connection to controlling biofilm formation. The findings are important for food safety because they show that *S. aureus* biofilm formation may be influenced by food processing circumstances [4].

In vitro, iron-restricted growth conditions are used to promote the formation of Staphylococcus aureus biofilms. In this research, we demonstrated that Emp and Eap are crucial in *S. aureus* Newman's biofilm formation when low iron is present. Eap and Emp are secreted proteins that are non-covalently attached to the surface of S. aureus cells. They have been earlier linked to several pathogenesis-related aspects of S. aureus. Here, we demonstrate that growth in a low-iron medium, which mimics the milieu found in vivo, induces the transcription of these crucial virulence factors. Our findings demonstrate that Fur is not necessary for iron control of Eap and Emp. However, in low-iron conditions, Fur is necessary for the complete induction of eap and emp expression. In this research, we showed that Sae, Agr, and SarA are also necessary for low-iron-induced biofilm formation. Sae and Agr are necessary for Emp and Eap expression, and consequently for the formation of biofilms, in iron-restricted growth conditions, whereas SarA seems to play a less important role. We also demonstrated that in iron-restricted growth circumstances, the ica operon must be expressed for biofilm formation. We proved that the crucial multifunctional virulence determinants eap and emp must express themselves for ica to work [5].

The human pathogen *Staphylococcus aureus* creates biofilm on catheters and surgical devices. In their previous research, the authors demonstrated that 1, 2, 3, 4, 6-penta-O-galloyl-D-glucopyranose (PGG) prevents the initial attachment of S. aureus cells to a solid surface and lowers the production of polysaccharide intercellular adhesin. (PIA). Our MALDI-TOF mass spectrometric and cDNA microarray research shows that PGG therapy induces the expression of genes and proteins that are typically expressed under iron-limiting circumstances. PGG is a potent iron chelator that removes iron from the growth medium, according to a chemical test using the compound ferrozine. According to this research, adding FeSO4 to a medium containing PGG allows S. aureus SA113 to form biofilms and produce PIA once again. Using a semi-defined medium, BM, that includes an iron chelating agent, 2, 2′-dipyridyl, it is also possible to confirm that S. aureus SA113 needs iron to form biofilms. (2-DP). Similar to how PGG works, adding 2-DP to BM medium prevents the formation of biofilms, and adding FeSO4 to 2-DP-containing BM medium encourages the formation of biofilms. This study [6] uncovers an essential mechanism of S. aureus SA113 biofilm formation.

One of the key factors in the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) as a pathogen of infection linked to medical devices is biofilm formation. Methicillin-susceptible *S. aureus* (MSSA) can nevertheless produce biofilms in vitro, and these biofilms are vancomycin-resistant. Therefore, it is urgent and necessary to conduct a study on the potential mechanisms of MSSA biofilm formation. Using RNA-seq technology, we examined gene expression patterns in biofilms after ursolic acid and resveratrol treatment using S. aureus ATCC25923 as the model strain. The findings demonstrated that only ursolic acid could prevent the growth of biofilms, in contrast to their application on the multi-drug-resistant MRSA biofilm. Six genes implicated in the formation of biofilms were examined for expression by qRT-PCR to validate the RNA-seq data. These data analyses showed that the absence of an accessory gene regulator (agr) function in MSSA meant that its biofilm formation process was distinct from that of MRSA. These results imply that S. aureus biofilms with dysfunctional agr may be more robust than those with functional agr. As a result, once a biofilm has formed, the illness caused by clinical MSSA may be resistant. To

understand the processes of biofilm formation in other clinical S. aureus, more research is required [7].

This study aimed to evaluate the *Staphylococcus aureus* biofilm formation and Nε-carboxymethyl-lysine generation ability under food heat processing conditions including pH (5.0–9.0), temperature (25□°C, 31□°C, 37□°C, 42□°C and 65□°C), NaCl concentration (10%, 15% and 20%, w/v) and glucose concentration (0.5%, 1%, 2%, 3%, 5%, 10%, w/v). By using PCR to find the atl, ica operon, sasG, and agr genes, the genetic makeup of S. aureus biofilms was discovered. Crystal violet and methyl thiazolyl tetrazolium staining techniques were used to measure the biomass and metabolic activity of the biofilm. Food heat processing conditions of 37 °C, pH 7.0, 2% w/v glucose concentration, and 10% w/v NaCl concentration were advantageous for S. aureus biofilm growth. Additionally, strong, moderate, and weak biofilms were all identified by optimized high-performance liquid chromatography-tandem mass spectrometry for free and bound N-carboxymethyl-lysine levels. Strong, intermediate, and weak biofilm strains of S. aureus exhibited a significant difference in N-carboxymethyl-lysine levels. According to this study, Staphylococcus aureus biofilm poses a biological and chemical risk to the environment around food processing [8].

Staphylococcus aureus's polysaccharide intercellular adhesin (PIA/PNSG) and icaADBC gene locus were newly discovered, but in vitro biofilm formation has only infrequently been observed. In this study we evaluated a tissue culture plate (TCP) assay and a tube test, as well as Congo red agar, using the two basic media trypticase soy broth (TSB) and brain heart infusion (BHI) broth with different sugar supplements for detection of biofilm formation in 128 ica-positive S. aureus isolates. In the TCP test, 57.1% of the S. aureus isolates showed a biofilm-positive phenotype under ideal circumstances. Strongly biofilm-producing strains showed good correlation between the tube test and the TCP test, but weak producers could not be reliably distinguished from biofilm-negative strains. Screening on Congo red agar showed a significant correlation with the TCP and the tube test for just 3.8% of the samples, so it is not advised for research on S. aureus biofilm formation [9].

The fluid shear levels that the opportunistic pathogen *Staphylococcus aureus* meets within the human host can affect whether the organism adopts a commensal interaction with the host or develops into a pathogen. S. aureus was examined for cellular responses that could affect its colonization and virulence in rotating-wall vessel bioreactors, which were used to produce a physiologically relevant, low-fluid-shear environment. S. aureus cells developed a novel attachment-independent biofilm phenotype and were fully encased in extracellular polymeric materials when grown in a low-fluid-shear environment. Low-shear-cultured cells showed slower growth and suppressed virulence traits, including decreased carotenoid production, increased oxidative stress sensitivity, and decreased survival in whole blood, when compared to controls. Alterations in metabolic pathways were indicated by transcriptional whole-genome microarray profiling.

Additional research on genetic expression showed Hfq's downregulation, which is consistent with some Gram-negative organisms' responses to low fluid shear. This research is the first to document an association between Hfq and fluid shear in a Gram-positive organism, indicating that prokaryotes of various structural types have a conserved evolutionary response to fluid shear.

Collectively, our results suggest S. aureus responds to a low-fluid-shear environment by initiating a biofilm/colonization phenotype with diminished virulence characteristics, which could lead to insight into key factors influencing the divergence between infection and colonization during the initial host-pathogen interaction [10].

CONCLUSION

Staphylococcus aureus chronic biofilm-associated infections frequently result in substantial increases in morbidity and mortality, especially when connected to indwelling medical equipment. This has led to a significant amount of study into S. aureus biofilm formation and the molecular processes that underlie these multicellular structures' resistance to antibiotic therapy.

This review's goal is to provide an overview of our current knowledge of how S. aureus biofilms develop, with a particular emphasis on the description of a recently developed, five-stage biofilm development model and the mechanisms needed for each step.

Importantly, this model includes an alternate view of the processes involved in microcolony formation in S. aureus and suggests that these structures originate as a result of stochastically regulated metabolic heterogeneity and proliferation within a maturing biofilm population, rather than a subtractive process involving the release of cell clusters from a thick, unstructured biofilm.

It is important to note that it is suggested that this novel model of biofilm development entails the genetically programmed generation of metabolically distinct cell subpopulations, resulting in a population as a whole that is better able to adapt to quickly changing environmental conditions.

REFERENCES:

[1] H. Lade *et al.*, "Biofilm formation by staphylococcus aureus clinical isolates is differentially affected by glucose and sodium chloride supplemented culture media," *J. Clin. Med.*, 2019, doi: 10.3390/jcm8111853.

[2] S. Croes, R. H. Deurenberg, M. L. L. Boumans, P. S. Beisser, C. Neef, and E. E. Stobberingh, "Staphylococcus aureus biofilm formation at the physiologic glucose concentration depends on the S. aureus lineage," *BMC Microbiol.*, 2009, doi: 10.1186/1471-2180-9-229.

[3] D. E. Moormeier and K. W. Bayles, "Staphylococcus aureus biofilm: a complex developmental organism," *Molecular Microbiology*. 2017. doi: 10.1111/mmi.13634.

[4] T. M. Rode, S. Langsrud, A. Holck, and T. Møretrø, "Different patterns of biofilm formation in Staphylococcus aureus under food-related stress conditions," *Int. J. Food Microbiol.*, 2007, doi: 10.1016/j.ijfoodmicro.2007.02.017.

[5] M. Johnson, A. Cockayne, and J. A. Morrissey, "Iron-regulated biofilm formation in Staphylococcus aureus newman requires ica and the secreted protein Emp," *Infect. Immun.*, 2008, doi: 10.1128/IAI.01635-07.

[6] M. H. Lin, J. C. Shu, H. Y. Huang, and Y. C. Cheng, "Involvement of iron in biofilm formation by staphylococcus aureus," *PLoS One*, 2012, doi: 10.1371/journal.pone.0034388.

[7] X. Tan *et al.*, "Transcriptome analysis of the biofilm formed by methicillin-susceptible Staphylococcus aureus," *Sci. Rep.*, 2015, doi: 10.1038/srep11997.

[8] J. Miao *et al.*, "Biofilm Formation of Staphylococcus aureus under Food Heat Processing Conditions: First Report on CML Production within Biofilm," *Sci. Rep.*, 2019, doi: 10.1038/s41598-018-35558-2.

[9] J. K. M. Knobloch, M. A. Horstkotte, H. Rohde, and D. Mack, "Evaluation of different detection methods of biofilm formation in Staphylococcus aureus," *Med. Microbiol. Immunol.*, 2002, doi: 10.1007/s00430-002-0124-3.

[10] S. L. Castro, M. Nelman-Gonzalez, C. A. Nickerson, and C. M. Ott, "Induction of attachment-independent biofilm formation and repression of hfq expression by low-fluid-shear culture of Staphylococcus aureus," *Appl. Environ. Microbiol.*, 2011, doi: 10.1128/AEM.00175-11.

CHAPTER 23

BIOFILM DEVELOPMENT ON VARIOUS METAL SURFACES

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ABSTRACT

Due to their direct connection to the early failing of metal components, microbiological fouling, and microbiological corrosion have been the focus of extensive research for many years.

Sulfate-Reducing Bacteria (SRB)-induced corrosion is one of the most common types of microbiologically induced corrosion. (MIC). In terms of surface properties, metal/water interface structure, and chemical species, the existence and activity of SRB create an environment that is drastically different from bulk seawater, which causes localized corrosion of metal material mechanisms. Microorganisms that are pertinent to corrosion speed up corrosion due to their presence, excreted metabolites, and exopolymeric materials. At the metal/biofilm interface, microbial biofilm affects the surface responses. The development of biofilms and microbial adhesion is strongly influenced by surface properties, including homogeneity of the oxide layer, excess of alloying elements, pH, interference between exopolymers and metal ions, and interaction between aggressive metabolites and the metal surface. The microbes that affect corrosion are mostly harmful when they are sessile, embedded in biofilms, and much less when they are planktonic.

KEYWORDS:

Bacterial Biofilm, Corrosion Mic, Extracellular Matrix, Ferrous Alloys, Marine Environment.

INTRODUCTION

When building ships, bridges, and factories, metal alloys are frequently used as the primary raw material. Products made using known molding techniques are designed for long-term use in marine environments. Biofilms are created in marine habitats when microorganisms stick to metal surfaces. Complexes of microbes called biofilms are encased in an extracellular polymeric substance (EPS) matrix and have a three-dimensional structure. Extracellular matrix proteins and exogenous genes are moved through the EPS matrix within the biofilm, which promotes microbial development.

A well-developed biofilm also clings to metal surfaces tightly thanks to the extracellular polysaccharides of the EPS matrix, making it challenging to fully remove. The biofilm protects the microorganisms from the stressors brought on by alterations in the environment and chemical substances. As a result, inside the biofilm, a favorable environment for microbial growth forms, and the microbial community gradually grows to a high density. Microbiologically influenced corrosion (MIC) in biofilms produced on metal surfaces is brought on by a variety of microbial factors, including metal oxidation by electron transfer from the metal surface to bacteria and accumulation of corrosive metabolites. In the marine environment, MIC poses significant issues for metal infrastructure, and in 2013, it was estimated to have cost the global economy $2.5 trillion, or roughly 3.4% of GDP. MIC evaluations in the maritime environment have been documented for low-alloy steel, carbon steel [5], and stainless steel.

Understanding how plastics behave in the environment is essential to create plastic remediation strategies. The two primary processes that can affect how polymers behave and how long they last in the environment are thought to be physicochemical weathering (UV-induced, thermal, etc.) and microbial biofilm development. Particularly microbial processes can affect how plastics behave in the environment by controlling how they engage with the biota there. While the negative effects of terrestrial and aquatic plastic debris on the local biota are frequently recorded, little is known about how plastic interacts with these organisms. To comprehend the behavior and general fate of plastics, it is essential to comprehend the relationship between microbes and biofilm formation on plastics [1]. The aerobic heterotrophic bacteria species that make up the immature biofilm typically have a great deal of microbial variety, particularly those belonging to the phyla Proteobacteria and Bacteriodetes (Figure.1A). The general theory is that the aerobes use the dissolved oxygen in the area around them to create a chemical gradient that makes the interior zone anoxic and promotes the development of anaerobic species. (Figure. 1B).

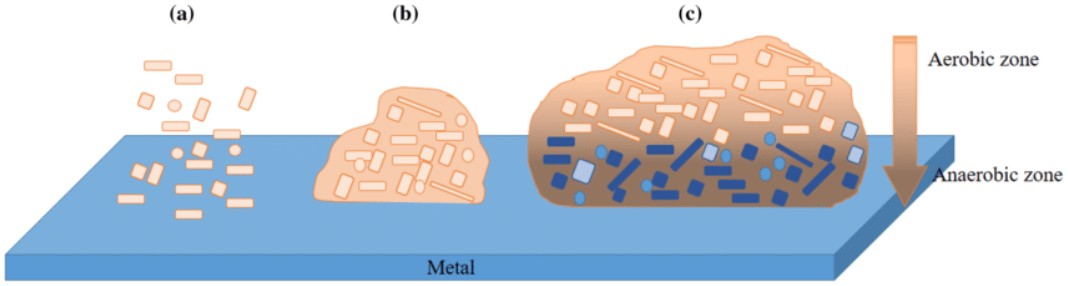

Figure 1: Steps involved in biofilms growth: Diagram showing the different steps involved in the growth of the biofilms on the metal surface (research gate).

The natural surroundings will have an impact on how the biofilm develops. The species that will make up the mature biofilm will depend on variables such as temperature, salinity, pH level, availability of nutrients, exogenous inputs of new species, and, most importantly, the composition of the metal attacked. Different microbial metabolic groups operate in different ways on the metal in a biofilm that forms over iron and steel infrastructures. Each microbial group performs metabolic processes, such as iron oxidation, iron reduction, and sulfate reduction that co-aggregate in strata or locations other than metal surfaces. (Figure. 1C). One of the causes of this is the abundance of microhabitats, which are made up of various redox potential sites and countless chemical substances in corrosive biofilms.

Development stages of a biofilm on a metal surface. On a metallic surface, attachment is started by planktonic cells. Extracellular material, which will eventually make up EPS, is created after the establishment of cells on the surface. The biofilm's interior has adequate oxygen levels for the presence of aerobic bacteria at this point of development because the corrosion process has not yet started. The most superficial layer of the biofilm still contains significant levels of oxygen while the innermost layer in contact with the metal matures with a chemical oxygen gradient, showing an anoxic environment. Anaerobic microbes linked to metal corrosion processes are common at this stage [2].

The two types of bacterial participation in the corrosion process are direct (where bacteria directly affect the rate of anodic and cathodic reaction) and indirect (where bacteria produce acidic metabolites that speed up the materials' corrosion process). Bacterial activities result in deposits building up and bacterial biofilms developing on ferrous alloys, which in turn causes serious corrosion attacks. Localized corrosion like pitting corrosion and crevice corrosion is primarily caused by MIC. Both pitting and crevice corrosion are examples of localized corrosion, which means that they only affect a small portion of the surface and have a higher

erosion rate than uniform corrosion. The crevice corrosion typically takes place in a crack that is only a few micrometers wide. These fissures are typically brought on by outside elements that create a crevice on the surface of the substance, such as insulation and paint scraps. The initiation sites for pitting corrosion are typically spots of microbial colonization and chloride accumulation that quickly penetrate the barrier oxide layer covering the metal surface. Additionally, selective dissolution, which happens when one of the components dissolves more quickly than other components, is another method to start pitting corrosion. As time goes on, this localized dissolution causes a pit to develop on the metal's surface. According to earlier studies, bacterial biofilm-induced increases in cathodic response rate facilitated the spread of crevice corrosion. When mesophilic microorganisms and biofilms are present, stainless steel alloy has been found to experience severe crevice corrosion.

Additionally, it has been noted that the likelihood of microorganisms in marine water and the development of their biofilm on the surface of the substance raised the risk of pitting corrosion. On the subject of how microorganisms and ferrous alloys interact, a great deal of study has been done. (Figure.2) lists the key conclusions reached by scholars studying the MIC of ferrous alloys. Additionally, various MIC mitigation strategies have been put forth by researchers working in this field. Below are a few of the suggested remedies: a) putting an end to the iron-oxidizing bacteria, b) preventing bacteria from coming into touch with the metal substrate, c) repairing the metal substrate with a non-metallic option that is resistant to MIC, (d) the use of biocides, (e) mechanical cleansing methods, and (f) the addition of antibacterial substances like copper and zinc.

Materials	Authors	Microbes	Effects
carbon steel	Hamza et al. [4]	*P. aeruginosa*	Biofilm formation, corrosion damages, high corrosion rate
304 SS	Hamza et al. [5]	*P. aeruginosa*	Biofilm formation, formation of differential aeration cells, pitting corrosion
Titanium	Khan et al. [7]	*P. aeruginosa*	Biofilm formation, pitting corrosion
Carbon steel	Javed et al. [8]	SRB	Biofilm formation, EPS secretion, pitting corrosion attacks, high corrosion rate
Iron based oil and gas pipelines	Dennis et al. [22]	SRB	Production of corrosive hydrogen sulfide, increased deterioration of iron
Low carbon steel (Ship ballast tank)	Heyer et al. [26]	Slime forming bacteria, sulfur oxidizing (SOB), iron oxidizing bacteria (IOB) and sulphate reducing bacteria (SRB)	Increase pitting corrosion, high corrosion rate
316L stainless steel	Dong et al. [27]	*Acidithiobacillus caldus* SM-1	Development of dense biofilm, severe pitting corrosion, high corrosion rate
316L stainless steel	Tang et al. [28]	*Geobacter sulfurreducens* and *Geobacter metallireducens*	Direct electron transfer encouraged the corrosion of stainless steel
304 stainless steel	Zhang et al. [29]	*Desulfovibrio vulgaris*	Electron mediator increased corrosion, weight loss, pitting corrosion

Figure 2: Biofilms on a different surface: Diagram showing the different biofilms which grow on different metal surfaces (Metal).

LITERATURE SURVEY

Biocorrosion, also known as microbially influenced corrosion, is the word used to describe the metals' accelerated deterioration as a result of biofilms on their surfaces. Biocorrosion's intricate mechanisms are still not fully known. Recent studies into biocorrosion have concentrated on how biomineralization processes on metallic surfaces affect electrochemical reactions at the biofilm-metal interface as well as the effects of extracellular enzymes acting within the biofilm matrix. Biocorrosion, also known as microbially influenced corrosion, is the word used to describe the metals' accelerated deterioration as a result of biofilms on their surfaces. Biocorrosion's intricate mechanisms are still not fully known. Recent studies into biocorrosion have concentrated on how biomineralization processes on metallic surfaces affect electrochemical reactions at the biofilm-metal interface, as well as the effects of extracellular enzymes acting within the biofilm matrix [3].

Pseudomonas fluorescens adherence to nano- and micro engineered surfaces was investigated. According to the findings, these bacteria created distinct aggregates on nanosized, granular gold substrates that were haphazardly oriented. These collections of bacteria are aligned ensembles, some of which are highly elongated. Bacterial alignment and cell-to-cell adhesion were prevented on ordered manufactured surfaces, so this type of biological structure was not present. Importantly, between bacteria attached to the ordered nano/microstructures and the haphazardly ordered surfaces, variations in cell morphology, length, orientation, and flagellation were seen. The ramifications of the findings concern both the biocontrol of soil ecosystems and the design of engineered surfaces to improve (nanostructured filters) or inhibit (medical implants and industrial biofouling) bacterial colonization on the surfaces [4].

Using a variety of microscopy techniques, biofilms formed in various environments and under field or laboratory circumstances on naturally occurring and man-made surfaces have been thoroughly studied at varying stages of development. Except for scanning electron microscopy (SEM), the preponderance of these techniques, while qualitative, does not reveal how the biofilm affects the underlying substratum. A powerful tool for characterizing the qualitative and quantitative aspects of biofilm/substratum interactions, in comparison, is atomic force microscopy (AFM). The application of AFM for the study of bacterial biofilms is outlined in this communication, with a focus on particular studies involving metallic surfaces like stainless steel and copper alloys in freshwater and marine environments [5].

Economic losses from corrosion are significant. The currently popular corrosion control methods have the drawbacks of being costly, susceptible to environmental restrictions, and occasionally ineffective. According to studies, microbial corrosion prevention is a typical occurrence. The current review summarizes recent developments in this innovative strategy for controlling corrosion by creating biofilms of helpful bacteria. The possible mechanisms may involve: (1) removal of corrosive agents (such as oxygen) by bacterial physiological activities (e.g., aerobic respiration), (2) growth inhibition of corrosion-causing bacteria by antimicrobials generated within biofilms [e.g., sulfate-reducing bacteria (SRB) corrosion inhibition by gramicidin S-producing *Bacillus brevis* biofilm], (3) generation of the protective layer by biofilms (e.g., *Bacillus licheniformis* biofilm produces on the aluminum surface a sticky protective layer of γ-polyglutamate). Advances in research at the intersection of corrosion engineering and biofilm biology are necessary for the effective application of this innovative strategy [6].

Surfaces draw bacteria to them. Biofilms are created as a result of their surface adherence, binary fission, and the exopolymer that results. These biofilms are made up of bacteria

embedded in a framework of exopolysaccharide glycocalyces. Biofilms make up a third physical component in addition to the bulk fluid and the surface. The creation of metabolically dependent consortia is aided by the proximity of the bacterial cells in the biofilm matrices. A heterogeneous system is created at the surface that has been colonized by these microbial communities' chemical and physical actions. Effective anodes and cathodes can form at nearby locations on the surface as a result of metabolites produced at particular points on the surface. In this manner, the fouling of a surface by the development of a bacterial biofilm enables a focused attack on that surface. This pit development is typical of bacterial surface processes that include metal corrosion and dental decay. In this study, we look at focal bacterial attacks, biofilm formation, and bacterial adhesion to surfaces that have been colonized. The fouling of biological surfaces and pathogenic biofilms, except caries formation, are outside the purview of this paper [7].

In many economic sectors, metal corrosion is a significant worldwide concern. Only in the US, declines in values amounting to about 3% of GDP are brought on by the deterioration of metal surfaces. The majority of corrosion processes documented in various environments occur in marine environments. The corrosion of several metallic alloys is said to be favored by the marine environment, which damages structures used to build ships, ports, hydrocarbon pipes, and other things. Although chemical corrosion is the type that is most frequently mentioned in these environments, studies have shown that microorganisms play a role in both direct corrosion processes and the acceleration or impact of the corrosive action by creating intricate biofilms. These features foster the growth of microbes that corrode metal surfaces and leave pitting and crevices behind. Currently, biocorrosion study uses a variety of technicians, including those who specialize in electronic microscopy and DNA sequencing. These methods have made the dynamic process of biofilm structure creation more clear, enabling comprehension of the succession of various species during the structure's evolution. It will be easier to evaluate strategies to stop or slow down the deterioration of metallic structures in marine environments if we have a clearer grasp of how this interaction between biofilm and metallic surface happens [2].

Based on research previously documented by various authors as well as work completed by the author with collaborators from other institutions and his graduate students at CEEL, this review discusses various examples of the interaction of bacteria and metal surfaces. Traditional thinking has held that "microbiologically influenced corrosion" (MIC), which refers to the interplay of bacteria with metal surfaces, always results in higher rates of corrosion. More recently, it has been discovered that numerous bacteria can slow down the corrosion of various metals and alloys in a variety of corrosive settings. For instance, it has been discovered that specific strains of Shewanella can stop mild steel from rusting, brass from tarnishing, and Al 2024 from pitting in artificial saltwater. Corrosion has been seen to reappear after the biofilm was destroyed by the addition of antibiotics. Since it was discovered that the corrosion potential of Ecorr became more positive in the presence of Bacillus subtilis but more negative in the presence of Shewanella ana and algae, it appears that the mechanism of corrosion protection differs for various bacteria. These results were applied to a preliminary investigation of the bacterial battery in which *Shewanella oneidensis* MR-1 was added to an Al 2024 and Cu-containing cell in a growing medium. It was discovered that this cell's power production grew steadily over time. Bacteria oxidize the fuel and transfer electrons straight to the anode in the microbial fuel cell (MFC). In preliminary research, EIS has been used to describe the anode, cathode, and membrane properties of an MFC containing *Shewanella oneidensis* MR-1 under various operating circumstances. Using potentiodynamic sweeps, cell voltage (V)—current density (i) curves were produced. A MFC's current production has been tracked under various experimental circumstances [8].

The viewpoint on bacterial biofilms' ability to prevent corrosion is presented. It has been documented that biofilms can prevent general rust on mild steel, copper, aluminum, and stainless steel. The mechanisms most frequently cited for the inhibition are the formation of a diffusion barrier to corrosion products that stifles metal dissolution, consumption of oxygen by respiring aerobic microorganisms within the biofilm causing a diminution of that reactant at the metal surface, production of metabolic products that act as corrosion inhibitors (e.g., siderophores) or specific antibiotics that prevent the proliferation of corrosion-causing organisms (e.g., sulfate-reducing bacteria), and formation of passive layers that are unique to the presence of microorganisms. The viewpoint will go over how biofilms are created as well as the processes that are challenging to predict and manage [9].

CONCLUSION

According to an analysis of the study studies conducted by various researchers in the MIC field, microorganisms like fungi, algae, and bacteria are responsible for the deterioration of different metallic alloys in various areas.

The majority of the microorganisms found in marine environments are corrosive, according to the study studies compiled, but in some instances, it has also been discovered that bacteria can prevent materials from corroding. MIC is influenced by bacterial biofilm and its biochemical processes. Ferrous metals may corrode as a result of microbes. Microorganisms play significant parts in the corrosion process, which is explained by MIC mechanisms such as concentration cells, metabolite-induced corrosion, as well as MIC based on bioenergetics and bio-electrochemistry. However, systematic studies of MIC mechanisms are still lacking as a result of the complexity and multidisciplinary character of MIC. To fully comprehend MIC processes, additional research into the genetic manipulation of corrosion-causing microorganisms is required.

REFERENCES:

[1] G. Bhagwat, W. O'Connor, I. Grainge, and T. Palanisami, "Understanding the Fundamental Basis for Biofilm Formation on Plastic Surfaces: Role of Conditioning Films," *Front. Microbiol.*, 2021, doi: 10.3389/fmicb.2021.687118.

[2] L. Procópio, "The role of biofilms in the corrosion of steel in marine environments," *World Journal of Microbiology and Biotechnology*. 2019. doi: 10.1007/s11274-019-2647-4.

[3] I. B. Beech and J. Sunner, "Biocorrosion: Towards understanding interactions between biofilms and metals," *Current Opinion in Biotechnology*. 2004. doi: 10.1016/j.copbio.2004.05.001.

[4] C. Díaz, P. L. Schilardi, R. C. Salvarezza, and M. F. L. De Mele, "Nano/microscale order affects the early stages of biofilm formation on metal surfaces," *Langmuir*, 2007, doi: 10.1021/la700650q.

[5] I. B. Beech, J. R. Smith, A. A. Steele, I. Penegar, and S. A. Campbell, "The use of atomic force microscopy for studying interactions of bacterial biofilms with surfaces," *Colloids Surfaces B Biointerfaces*, 2002, doi: 10.1016/S0927-7765(01)00233-8.

[6] R. Zuo, "Biofilms: Strategies for metal corrosion inhibition employing microorganisms," *Applied Microbiology and Biotechnology*. 2007. doi: 10.1007/s00253-007-1130-6.

[7] H. M. Lappin-Scott and J. William Costerton, "Bacterial biofilms and surface fouling," *Biofouling*, 1989, doi: 10.1080/08927018909378120.

[8] F. Mansfeld, "The interaction of bacteria and metal surfaces," *Electrochimica Acta*. 2007. doi: 10.1016/j.electacta.2007.05.006.

[9] B. Little and R. Ray, "A perspective on corrosion inhibition by biofilms," *Corrosion*, 2002, doi: 10.5006/1.3277632.

CHAPTER 24

PRESENTING THE DIVISION OF LABOR IN BIOFILMS ALONG WITH THE DIFFERENTIATION OF CELL ECOLOGY

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ABSTRACT:

The idea of bacterial multicellularity, which was first proposed almost two decades ago, is now acknowledged as a fundamental aspect of bacterial metabolism. Division of work and cell-to-cell communication are found to be commonplace across all bacterial species, supporting the idea that bacteria are more complex than simple unicellular, disorganized, selfish organisms. Bacteria can organize themselves into intricate communities where cells can differentiate in a spatiotemporal manner, utilizing extracellular cues to regulate the expression of particular genes necessary for structural development. Despite the significant advancements made in the field in recent years, little is known about the molecular processes that control bacterial multicellularity and the formation of biofilms, and this area of study continues to be of great interest.

KEYWORDS:

Cell Types, Division Labor, Surface Sensing, Spatial Patterns, Wild Type.

INTRODUCTION

The ability of the evolutionary process to build is one of its most amazing traits. A primordial soup of organic compounds developed over billions of years into the current stage of life. The evolution of the first prebiotic cells, eukaryotes, multicellularity, and eusociality are just a few examples of transitions that have taken place throughout the natural history of our planet that best demonstrate this ability to build. There are several glaring parallels among these transitions. First, collaboration is how building progresses. To put it another way, previously autonomous biological components work together to form new organizational layers. For instance, multicellularity developed from cells that cooperate by sticking together, either through incomplete cell division or through aggregation, and organelles from microbes that participated in mutualistic interactions through endosymbiosis.

Major evolutionary transitions are also characterized by the division of work, in addition to cooperation. The term "diversity of labor" will be defined precisely below, but it can also be used broadly to refer to the specialization of people in carrying out various "tasks" during cooperative interactions. Multicellular development offers arguably the most striking illustration. Numerous specialized cell kinds make up multicellular organisms. (e.g., muscle cells, neurons, epithelia, etc.). Although these cells share the same genetic makeup, they have differentiated and arranged themselves into various physiological and morphological structures (such as organs), which together make up the individual [1].

Division of work among microbial assemblies is already known to occur, particularly within biofilms. Even a genetically clonal community can become divided into subpopulations with radically different behaviors in *B. subtilis* biofilms, for instance. In these biofilms, genetically similar cells differentiate into cells that are specialized for motility, matrix formation, and sporulation, all of which are crucial for the biofilm's success as a whole. However, how does the maintenance of such a system among people benefit from the costs and advantages of the

division of labor for each cell? The mechanisms by which the division of work is carried out in a biological system must be revealed. As an illustration, consider persistent microbial infections. Division of labor among microbes can increase their damage to us through synergy.

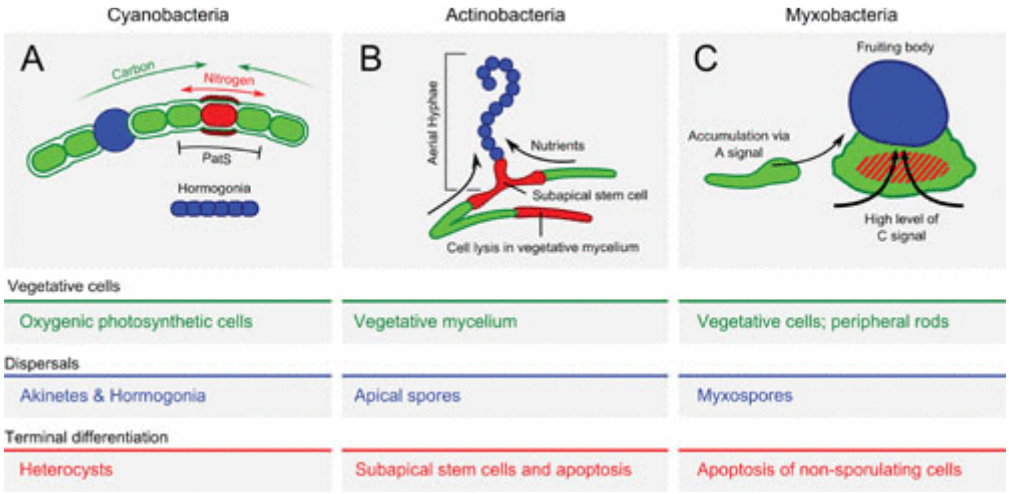

Figure 1: Division of labor in microorganisms: Diagramed showing the division of labor in different biofilms (ASM JOURNAL).

Staphylococcus aureus and *Pseudomonas aeruginosa* divide virulence duties in chronic wounds, making it more difficult for the immune system or antibiotics to suppress them. Similarly to this, it is believed that *P. aeruginosa* differentiation makes the infection more difficult to cure in the lungs of people with cystic fibrosis. As a result of the numerous unknowns and uncertainties regarding people, interactions, and the environment, it is difficult to disentangle how the division of labor occurs in natural settings. Concentrate on *B. subtilis* as a manageable system to elucidate the emergence and upkeep of the division of labor. In this system, the capacity to observe, manage, and manipulate subpopulations provides a direct method for mechanistically investigating the preservation of the division of labor. Specifically designed mutants are created to evaluate the effects of related genes, and fluorescent markers are used to track the expression of pertinent genes. They can investigate the genetic and phenotypic elements of labor division using this combination [2]. Pay close attention to the creation of the extracellular matrix, which is made up primarily of the protein TasA and the exopolysaccharide (EPS).

These elements are necessary for the biofilm's extracellular matrix to develop, but their production is expensive. To directly demonstrate that the wild-type strain does incur a production cost in comparison to the mutants that do not contribute to matrix production, researchers created mutant strains of *B. subtilis* that were deficient in either the ability to make EPS (Deps) or the TasA protein (DtasA). Furthermore, they discover that TasA and EPS are shared commodities. When combined in a culture, Deps and DtasA mutants produce a biofilm similar to that of the normal type, while mutants on their own are unable to do so. (Figure 1A). This demonstrates that matrix production can be split into two tasks and that subpopulations performing these two distinct tasks can still carry out the general function. The burden of matrix production is then divided between EPS producers and TasA producers in the wild-type population. They noticed three types of individuals in wild-type populations: matrix nonproducers, EPS producers, and generalists, which produce both EPS and TasA. They used fluorescent reporters to monitor the expression of EPS and TasA; instead of a

complete EPS-TasA divide (Figure 1B, bottom), they observed this split into three subgroups. (Figure 1B, top). This indicates that genes related to matrix production exhibit phenotypic variation in the wild-type biofilm [3].Differentiated tasks in the examples we will address below, such as the division of labor between vegetative growth and sporulation, are mutually incompatible and cannot be performed by a single cell at the same time. We then examine specific instances of divisions of labor using these generalized features before concentrating on Bacillus.

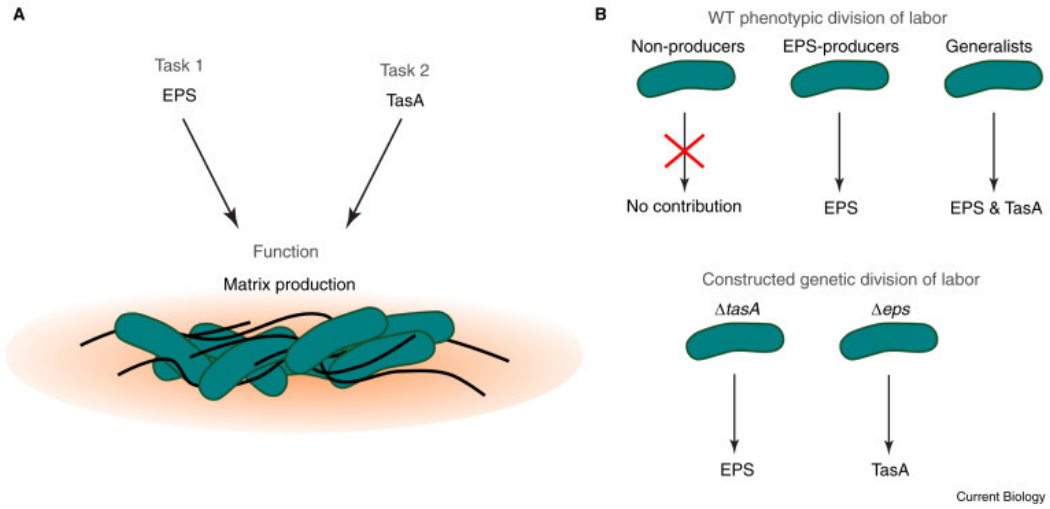

Figure 2: Division of labor: Diagramed showing the division of labor in the *bacillus subtilis* (cell press).

LITERATURE SURVEY

The ability of the evolutionary process to build is one of its most amazing traits. A primordial soup of organic compounds developed over billions of years into the current stage of life. The evolution of the first prebiotic cells, eukaryotes, multicellularity, and eusociality are a few transitions that took place during the natural history of our planet and serve as the best examples of this ability to build. There are several notable similarities among these transitions. First, collaboration is essential for building to advance. To put it another way, previously autonomous biological components work together to form new organizational layers. For instance, multicellularity developed from cells that cooperate by sticking together, either through incomplete cell division or through aggregation, from microbes that participated in mutualistic interactions through endosymbiosis. Major evolutionary shifts are also characterized by the division of work, in addition to cooperation. The term "diversity of labor" will be defined precisely below, but it can also be used broadly to refer to the specialization of people in carrying out various "tasks" during cooperative interactions. Multicellular development offers arguably the most striking illustration. Numerous specialized cell kinds make up multicellular organisms. (e.g., muscle cells, neurons, epithelia, etc.). Although these cells share the same genetic makeup, they have differentiated and arranged themselves into various physiological and morphological structures (such as organs), which together make up the individual [1].

In many bacterial species, the second messenger signaling molecule cyclic diguanylate monophosphate (c-di-GMP) regulates the switch from planktonic to biofilm development. In reaction to surface adhesion, *Pseudomonas aeruginosa* has two surface sensing systems that release c-di-GMP. According to current theories in the field, cells react uniformly by producing c-di-GMP after attaching to a surface. Here, we explain how the Wsp system

creates surface sensing heterogeneity, leading to the existence of two physiologically different cell subpopulations. One subpopulation, which acts as the originator of the first microcolonies, generates a biofilm matrix and has elevated c-di-GMP levels. The other subpopulation is surface motile and has low c-di-GMP, enabling surface exploration. We also demonstrate how the surface behavior of descendent cells is highly correlated with this heterogeneity. Our findings are taken together imply that *P. aeruginosa* participates in a division of labor following surface attachment that endures across generations, accelerating early biofilm formation and surface exploration [4].

A complex metabolic pathway can be taxing to the host if it is restricted to a single community, which lowers the system's total productivity. This limitation can be overcome by division of labor (DOL), where distinct populations execute various steps of the pathway, thus reducing the burden on each community. However, DOL decreases the efficacy of reactions by adding a transport barrier for metabolites and enzymes. It is still unclear how the possible advantage of DOL is determined by the trade-off between lowering burden and lowering reaction efficiency. We develop a general criterion for determining when DOL performs better than a single population through the analysis of various metabolic pathways. Our findings can aid in the logical design of metabolic pathways and offer information about how natural pathways function [5].

It is known that several bacterial processes, including biofilm formation or the development of reproductive structures, cause bacteria to differentiate into cells with unique phenotypic traits. These cell types represent a division of work because of their unique functions. However, it is unclear how bacteria create spatial arrangements of differentiated cells. Here, we investigate the variables that influence the spatial patterns of labor divides in colonies of *Streptomyces coelicolor*, a multicellular bacterium that can produce a wide range of antibiotics and intricate reproductive structures. (e.g., aerial hyphae and spores). We show that in *S. coelicolor* colonies, distinct waves of gene expression that radiate outwardly trigger the pathways for antibiotic biosynthesis and the formation of aerial hyphae. We also demonstrate how AdpA, a crucial activator in the developmental pathway, affects the spatiotemporal separation of these cell kinds. Importantly, expression in these pathways could be decoupled and/or disordered when we altered local gradients by cultivating rival microbes nearby or through physical disturbance. Finally, by including a siderophore, a product of these organisms, in the growth medium, the regular spatial organization of these cell types was partly recovered. Together, these findings suggest that physiological gradients and regulatory network design, two crucial elements that also influence patterns of cellular differentiation in multicellular eukaryotic organisms, are responsible for the spatial divisions of labor in *S. coelicolor*colonies [6].

The emergence of higher-order structures from interacting units is a crucial characteristic of biological systems. Examples include the development of tissues from individual cells and the intricate labor divisions in insect communities. However, little is known about how individual evolutionary competition impacts the biological organization. Here, we examine this connection in the context of bacterial biofilms, a concrete system well recognized for its higher-order architecture. We present a mechanistic model of cell growth at a surface and demonstrate how the tension between growth and competition for nutrients can account for the emergence of patterns in biofilms that have been experimentally observed. The maintenance of patterns requires cell cooperation, as we find when we apply our model to evolutionary simulations. In particular, natural selection supports energetically expensive spreading strategies, like polymer secretion, that concurrently lower productivity and disrupt the spatial patterns when different genotypes collide and compete. Our theory establishes a

formal connection between the potential for evolutionary conflict and higher-level patterning by demonstrating that both can result from the same collection of scale-dependent processes. Additionally, our analysis predicts an antagonistic relationship between evolutionary conflict and pattern formation: conflict promotes disorder [7]. This is in contrast to the previous theory.

In dynamic environments with a wide range of substrates in terms of variety and quantity, microorganisms obtain their energy and nutrients. Carbon catabolite repression is the regulatory mechanism in charge of arranging favored substrates in order of importance. (CCR). In the literature, there have been two major groups of CCR described. Model organisms like Escherichia coli have been used in theoretical and experimental studies of the best-described CCR strategy, known as classic CCR (cCCR). cCCR phenotypes are frequently, albeit occasionally incorrectly, used to extend universal fitness strategies. For instance, highly competitive microorganisms like pseudomonads whose distributions may be more widespread globally than those of E. coli have succeeded by using metabolic strategies that are almost the exact opposite of cCCR. Because the order of preferred substrates is nearly the opposite of that of cCCR, these organisms use a CCR strategy known as "reverse CCR" (rCCR). rCCR phenotypes do not allocate intracellular resources in a way that results in an overflow metabolism, favor organic acids over glucose, and may or may not choose preferred substrates to optimize growth rates. Even though the majority of microorganisms live in consortia, cCCR and rCCR have traditionally been interpreted in terms of monocultures. Here, we go over the fundamental principles of the two CCR strategies and look at these traits in the context of resource acquisition in consortia, a situation that undoubtedly had an impact on the development of cCCR and rCCR. For instance, cCCR and rCCR metabolism are nearly mirror images of one another. However, when viewed from the perspective of a consortium, the complementary qualities of the two strategies can reduce the likelihood of direct rivalry for energy and nutrients and instead create a cooperative division of labor [8].

CONCLUSION

Organisms must carry out a variety of duties to live and reproduce. However, trade-offs restrict how much time and money they can devote to each of these various procedures. One approach to addressing this issue is to focus on specific traits and collaborate with other organisms that can offer additional, complementary functions. Both parties gain from the interaction by reciprocally exchanging metabolites and/or services in this manner. Functional specialization or the division of labor are terms used to describe this phenomenon, which occurs frequently in nature and at all levels of biological structure. Additionally, various kinds of synergistic interactions have evolved among microorganisms. But it's not always clear whether a given example illustrates a case of labor division. By offering a set of standards that precisely describe the division of labor in microbial communities, we hope to close this gap. Additionally, we suggest a series of diagnostic tests to determine whether a specific encounter satisfies these requirements. Analysis shows that both intraspecific and interspecific interactions satisfy the criteria defining the division of labor, in contrast to how the word is typically used. Furthermore, rather than being social parasites, our analysis revealed non-cooperators of intraspecific public goods interactions to be growth specialists who collaborate with conspecific producers. This chapter discussed the identification of instances of the division of labor and inspire more in-depth analyses of this significant and common type of inter-microbial interaction by offering a conceptual toolbox.

REFERENCES:

[1] J. van Gestel, H. Vlamakis, and R. Kolter, "Division of Labor in Biofilms: the Ecology of Cell Differentiation," *Microbiol. Spectr.*, 2015, doi: 10.1128/microbiolspec.mb-0002-2014.

[2] B. Momeni, "Division of Labor: How Microbes Split Their Responsibility," *Current Biology*. 2018. doi: 10.1016/j.cub.2018.05.024.

[3] A. Dragoš *et al.*, "Division of Labor during Biofilm Matrix Production," *Curr. Biol.*, 2018, doi: 10.1016/j.cub.2018.04.046.

[4] C. R. Armbruster *et al.*, "Heterogeneity in surface sensing suggests a division of labor in pseudomonas aeruginosa populations," *Elife*, 2019, doi: 10.7554/eLife.45084.

[5] R. Tsoi, F. Wu, C. Zhang, S. Bewick, D. Karig, and L. You, "Metabolic division of labor in microbial systems," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 115, no. 10, pp. 2526–2531, 2018, doi: 10.1073/pnas.1716888115.

[6] V. M. Zacharia *et al.*, "Genetic network architecture and environmental cues drive spatial organization of phenotypic division of labor in streptomyces coelicolor," *MBio*, 2021, doi: 10.1128/mBio.00794-21.

[7] J. B. Xavier, E. Martinez-Garcia, and K. R. Foster, "Social evolution of spatial patterns in bacterial biofilms: When conflict drives disorder," *Am. Nat.*, 2009, doi: 10.1086/599297.

[8] H. Park, S. L. McGill, A. D. Arnold, and R. P. Carlson, "Pseudomonad reverse carbon catabolite repression, interspecies metabolite exchange, and consortial division of labor," *Cellular and Molecular Life Sciences*. 2020. doi: 10.1007/s00018-019-03377-x.

CHAPTER 25

FUTURE PERSPECTIVE OF BIOFILMS IN THE IN HEALTH, TECHNOLOGY, AND INDUSTRY

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ABSTRACT:

In many facets of daily living, including food production, biodegradation, the production of consumer goods, and genetic engineering, microbes are used. They are necessary for many different recipes. For example, the creation of coagulate cheese requires the presence of microorganisms. Through the creation of novel fuels, microbes can directly contribute to the creation of accessible renewable energy. Not only are novel methods of directly harnessing microbial energy being investigated, but microbial catalysts can also aid in the conversion of renewable resources into hydrocarbon fuels. In what is known as microbial biofuel cells, bacteria and other microbes may one day be used to create biofuel. The photosynthesizing bacteria are particularly intriguing in an energy setting. When subjected to light, they can produce electrical energy if they are connected to an electrode. In this chapter, we discussed the various aspects of the development of the biofilms used for human welfare and developing a polluted-free environment.

KEYWORDS:

Agricultural Significance, Genetic Material, Human System, Microbial Biofilms, Microbial Adhesion.

INTRODUCTION

Because of recent significant advancements in the tools available to study microbes, this is an exciting moment for microbiology. The discipline of molecular biology, which is the study of nucleic acids like DNA and RNA, has advanced to the point where many branches of microbiology now employ molecular tools. These instruments include DNA and RNA sequencing and manipulation, which have enabled microbiologists to manipulate microbial genomes and comprehend the nature of enzymes and the evolution of microorganisms (the genetic material of organisms).

The recent sequencing of the entire genome of the Yersinia pestis strain that caused England's Black Death epidemic, which wiped out the country's population in the 1300s, is an intriguing illustration of this. To reconstruct all of the bacterium's genes, DNA taken from the excavated remnants was meticulously sequenced. This revealed the strain's relationships to other Y. pestis strains that are still in existence. For instance, more bacteria and archaea species than anticipated have been found in recent ocean surveys, along with countless novel metabolic pathways. The microbes that live inside the human body are a prominent area of study for the microbiome. Everybody has a variety of microbes that naturally inhabit and are on their bodies, and these microbes may have a significant impact on human health and illness. Because there are more than ten times as many of these microbes as human body cells, microbiologists believe that this is the truth Microbes play a key role in the creation of food. Some soil microbes protect and fertilize the soil, which helps plants grow. Other soil microbes spoil food (spoilage), crops, and livestock. Still, other soil microbes directly create food through fermentation.

Microbes play a part in agriculture and food production that can affect crop health and possibly improve yield to help feed a growing world population, but we must also be careful because many agricultural methods require significant energy and environmental inputs. To achieve changes, researchers need to obtain scientific knowledge to promote the activities of microbes in the soil to reduce the use of energy-intensive chemicals like fertilizer; utilize microbes to help plants restore soil carbon; increase carbon storage by microbes on land and water; and engineer microbes to reduce the negative impacts of agricultural inputs. Infectious illnesses brought on by viruses, bacteria, fungi, and other microbes continue to afflict people, as evidenced by the current pandemic.

The most vulnerable populations to neglected tropical diseases like malaria and Ebola are those who reside in nations with limited resources and access to medical treatment. On the other hand, microbes produce a third of the medications we take, including many antibiotics (like penicillin), drugs that reduce cholesterol, and ones that fight cancer. Microbes are also the source of proteins used in vaccines and a variety of therapies, as well as factories for novel drugs created using recombinant DNA technology. Because they help with food processing and even produce some of the vitamins that are crucial for our health, gut microbes are important for good health. Microbes can enhance water quality by reducing pollution in the water. Children's diarrhea and cholera are two illnesses with high mortality rates that can be brought on by specific microbes in water.

Given that soil serves as the biggest water filter on the planet, it is crucial to preserve its microbial community's diversity, health, and integrity. On the other hand, some microorganisms, such as those that can degrade oil or other harmful toxins, have a positive effect on our water supplies. Through the creation of novel fuels, microbes can directly contribute to the creation of accessible renewable energy. Not only are novel methods of directly harnessing microbial energy being investigated, but microbial catalysts can also aid in the conversion of renewable resources into hydrocarbon fuels. To clean up soil, groundwater, and other contaminated areas of pollutants, researchers are developing microbial scrubbers. The Intergovernmental Panel on Climate Change (IPCC) predicts that changes to agricultural practices could reduce net carbon dioxide emissions by 100–1000 tons by the end of the twenty-first century, even though methane-producing microbes can also add to the input of greenhouse gases. An international supply chain that is distributed, sustainable, secure, and responsive to people's ever-changing requirements will be created by integrating microbes into a clean energy future.

Many businesses, including the production of food and pharmaceuticals, depend on microbes (Figure.1). As previously mentioned, there are many advantages to using microbes to transform renewable resources into energy, fuels, and chemicals. A "green bioeconomy" built on these skills has been made possible thanks to advances in genomics. It will make improvements in genomics, systems and synthetic biology, computational sciences, machine learning, and tech analysis to use microorganisms in a green bioeconomy. In such a future, agricultural productivity and product quality might improve, and a circular economy that recycles abundant materials might emerge.

Microbes are vital for living on land and in the water because they play a significant role in both ecosystem health and disease. As many bacteria are present in one gram of soil as there are humans on the planet. By making plants more tolerant of drought, defending them against disease, and giving them the nutrients they need to develop, soil bacteria improve the health of our crops. The decline of biodiversity in water, soil, land, and air can be halted by paying attention to the microbial sciences.

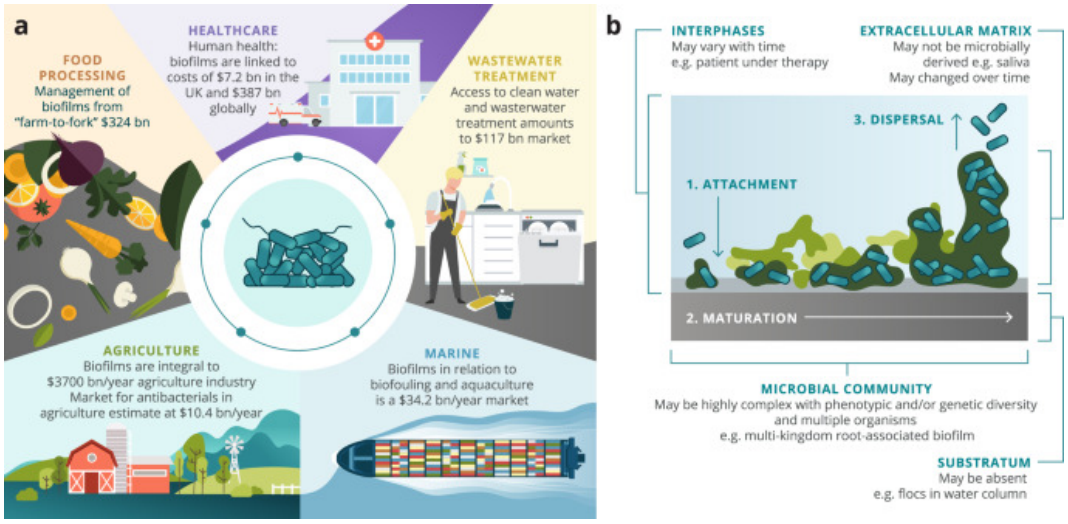

Figure.1: Biofilms and human: Diagramed showing the outline of the interaction of the biofilms with the human (Nature).

LITERATURE SURVEY

Due to the important functions that microbial biofilms play in the environment, industry, and human health, this topic is very interesting. Our knowledge of the structure and development of biofilms has improved as a result of developments in biochemical and molecular techniques. As a result of their enormous potential for crop production, protection, and improvement, biofilms in agriculture are recently getting attention. Previously, research on biofilms had a strong emphasis on the health and industrial sectors. In addition to improving soil fertility, biofilms are crucial for the colonization of surfaces such as soil, plant roots, or plant stems because they allow for growth in the targeted niche. Even though reports on microbial biofilms, in general, are available, little is known about how agriculturally significant microorganisms (bacteria, fungi, and bacterial-fungal) create biofilms and how they interact with the ecosystem. Improved knowledge of agriculturally significant bacterial-fungal communities and their relationships can have a variety of effects on things like bioremediation, soil quality, plant nutrition, and plant protection. Having a better understanding of the elements and genes involved in biofilm formation will aid in creating more efficient agricultural practices that are environmentally favorable and sustainable. With a focus on agriculturally significant microbial biofilms, the current review brings together basic aspects of biofilms concerning their formation, regulatory mechanisms, genes involved, and their application in various fields [1].

The characteristics of microbes in biofilms, which are highly organized and complex organisms that vary fundamentally from those of microbes in planktonic suspensions, are fundamentally different. Root canal diseases are caused by biofilm. Disinfection of the root canal system is very difficult due to the complexity and variability of the system as well as the multi-species character of the biofilms. The most significant reason for root canal therapy failure appears to be microbial persistence, which may also affect pain and quality of life. A chemo-mechanical procedure is used to remove biofilm, and it involves specialized tools, disinfectant compounds in the form of irrigants, and/or intracanal medications. Characterization of root canal biofilms and clinical techniques to disrupt the biofilms in addition to microbial eradication have been the main topics of endodontic study. In this narrative review, we talk about how bacteria biofilms affect endodontic treatment and examine the research on how root canal disinfectants and disinfectant-activating techniques affect biofilm removal [2].

Biofilm models are frequently used as study tools to find and close knowledge gaps in biofilm processes as well as simulation tools in engineering applications. Recent experimental evidence of biofilm heterogeneity calls into question the viability of the simplifying assumptions that engineering models depend on to be useful. On the other hand, research models are becoming more intricate and employ cutting-edge computational tools to mathematically explore what influences the population dynamics and structural heterogeneity of biofilms. Examining the significance of three-dimensional heterogeneities to the traditional biofilm models' predictive power is one of the objectives of advanced models. In addition, when researching a variety of biofilm-related events, biofilm models are employed to assess experimental findings. A specialized group was assembled to assess the current state and choose the future course of biofilm modeling study because of the variety of biofilm models' applications and the various methods modelers have used recently. The educational institutions of researchers and technicians on the basics of biofilm models, the development of mathematical models for real-time control of biofilm procedures, and the capacity to "engineer" the biofilm structure and operate (or achievement) were discovered as the most essential goals for the useful application of biofilm models. Biofilm models are used in mathematical research to better comprehend the structure and population dynamics of biofilms. A modeling study was found to require the evaluation of parameter sensitivity in various models. The group decided to start a cooperative project to compare and contrast the existing modeling approaches as a result of this meeting. Such a comparative study will improve our comprehension of biofilm processes and mathematical frameworks and will make it easier for scientists and engineers engaged in biofilm research to use biofilm models in the future [3].

Electrochemically active microorganisms can produce electroactive biofilms (EABFs), which have a wide range of possible uses in the production of bioenergy and chemicals. The output and effectiveness of the conversion processes can be significantly impacted by the electroactivity of biofilms. This study evaluates how process and design factors affect the development and behavior of biofilms in bioelectrochemical systems. (BESs). The function of planktonic and biofilm-forming microbes in BESs is first compared. To find assessment gaps and possible future modeling roles, the connection between electrochemical performance and operating parameters is also investigated. In a similar vein, we discuss the current state of knowledge regarding the processes by which electroactive biofilms transfer electrons as well as how the electrical conductivity of the exopolymeric components of the biofilms affects BES performance. Also reviewed is the present state of cathodic biofilms. To increase BES performance to the point required for commercial consideration, complementary strategies that use process control to optimize EABF composition and biomass density while minimizing mass transfer effects and changes to system design parameters are likely essential. Finally, future research needs that enable better understanding and optimization of the performance of EABFs are outlined [4].

In May 2019, 29 scientists with expertise in various subdisciplines of biofilm research got together in Leavenworth (WA, USA) at an event designated as the '2019 Biofilm Bash'. This unofficial two-day gathering's objectives were to first identify knowledge gaps and then to suggest methods for the biofilm community to close them. The meeting was structured around six topics that addressed the key issues raised by the hosts and participants. The current paper provides a summary of the results of these discussions. We understand that these opinions only cover a small portion of what is going on in the field and that we unavoidably missed out on some crucial new ideas and study areas. However, we remain optimistic that this report will spark debate and contribute to the development of fresh ideas for how we can progress in our field [5].

One of the most common causes of nosocomial infections, *Enterococcus faecalis* and *Enterococcus faecium* are notorious for their antibiotic resistance. They often result from biofilm-mediated infections linked to implanted medical devices or endocarditis and produce infections that are challenging to treat. Physical removal of devices or contaminated tissue is frequently required but frequently not feasible due to biofilms' resistance to antibiotics and phagocytosis. There are currently no therapeutically usable substances that break down biofilms. We address all known structural and regulatory genes involved in enterococcal biofilm formation in this review, along with compounds that have been investigated to prevent biofilm formation and potential drug targets for infections caused by enterococcal biofilms [6].

Due to the widespread intake of produce over the past three decades, outbreaks of food-borne pathogens associated with produce have significantly increased. Produce's susceptibility to microbial attack and the development of biofilms make a paradigm of food safety for produce essential. Decontaminating the bacteria in biofilms needs more focus because they endanger the public's health. To gain new knowledge about food safety, this review will concentrate on outbreaks, attachments, quorum sensing, biofilm formation, resistance to sanitizers and disinfectants, and current and emerging control methods for fresh and minimally processed produce. The development of a protective environment that is impervious to cleaning and disinfection is one of the effects of biofilms on produce. We'll quickly discuss alternative strategies for preventing the growth of biofilms on produce and point out any areas that require more study [7].

Research methods for biofilm are categorized by several studies, including those of microbial diversity and species, microbial proteins like enzymes, and microbial activity like metabolic activity. The genome, proteome, and metabolome are all components of biology's hierarchical structure, with the metabolome serving as the ultimate product of biological function. A novel approach to biological research in the twenty-first century is metabolome analysis, which is the complete analysis of the metabolome. Oral biofilm samples are too small to analyze the metabolome using conventional techniques, even though the stratified structure of biofilm research correlates to the biological hierarchy and the analysis of microbial activity, particularly metabolic activity, is similar to metabolome analysis. Recently, a novel tool that combines time-of-flight mass spectrometry (MS) and capillary electrophoresis (CE) has been created, enabling metabolomic investigation of the central carbon metabolic pathways (specifically, the TCA cycle, pentose phosphate pathway, and EMP pathway) in oral biofilm. We examined the metabolome profiles of oral biofilm using CE-MS after oral rinsing with glucose in vivo and assessed the effects of mouth rinsing with fluoride and xylitol. The findings were somewhat in line with earlier in vitro data from two distinct bacterial strains, Streptococcus and Actinomyces, but novel details describing the metabolic characteristics of oral biofilm were also discovered. The functional characteristics of oral biofilm in vivo will be revealed by this metabolomic approach, possibly revealing new details about the makeup of oral biofilm in both health and disease [8].

CONCLUSION

The most recent and upcoming advances in microbial biotechnology and bioengineering: microbial adhesion/biofilms in medical settings, microbial adhesion/biofilms in agriculture, and microbial adhesion/biofilm in the environment and industry are the three parts of the book Microbial Biofilms. The chapters cover human infections, microbial communication during the biofilm mode of development, host defense and antimicrobial tolerance, and more. They also cover adhesion and biofilm formation by pathogenic microbes on tissue and indwelling medical devices. Other parts discuss the biofilms of microbes that are beneficial to

agriculture and the environment, including how biofilms develop on plants, in soil, and aquatic environments. Finally, the most recent findings from scientific studies on microbial adhesion and biofilm development in the environment and business are discussed.

REFERENCES:

[1] K. Velmourougane, R. Prasanna, and A. K. Saxena, "Agriculturally important microbial biofilms: Present status and future prospects," *Journal of Basic Microbiology*. 2017. doi: 10.1002/jobm.201700046.

[2] P. Neelakantan *et al.*, "Biofilms in Endodontics—Current status and future directions," *International Journal of Molecular Sciences*. 2017. doi: 10.3390/ijms18081748.

[3] D. R. Noguera, S. Okabe, and C. Picioreanu, "Biofilm modeling: Present status and future directions," in *Water Science and Technology*, 1999. doi: 10.1016/S0273-1223(99)00178-X.

[4] A. P. Borole, G. Reguera, B. Ringeisen, Z. W. Wang, Y. Feng, and B. H. Kim, "Electroactive biofilms: Current status and future research needs," *Energy and Environmental Science*. 2011. doi: 10.1039/c1ee02511b.

[5] T. Coenye, B. Kjellerup, P. Stoodley, and T. Bjarnsholt, "The future of biofilm research – Report on the '2019 Biofilm Bash,'" *Biofilm*, 2020, doi: 10.1016/j.bioflm.2019.100012.

[6] F. L. Paganelli, R. J. Willems, and H. L. Leavis, "Optimizing future treatment of enterococcal infections: Attacking the biofilm?," *Trends in Microbiology*. 2012. doi: 10.1016/j.tim.2011.11.001.

[7] I. K. Jahid and S. Do Ha, "A review of microbial biofilms of produce: Future challenge to food safety," *Food Science and Biotechnology*. 2012. doi: 10.1007/s10068-012-0041-1.

[8] N. Takahashi, J. Washio, and G. Mayanagi, "Metabolomic approach to oral biofilm characterization-A future direction of biofilm research," *Journal of Oral Biosciences*. 2012. doi: 10.1016/j.job.2012.02.005.