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MICROBIAL PHYSIOLOGY AND METABOLISM

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CHAPTER 1

MICROBIOLOGY: AN OVERVIEW OF THE INVISIBLE WORLD

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ABSTRACT:

The area of biology known as microbiology is concerned with the study of microbes and how they affect people and other living things. The study of microorganisms encompasses the unicellular or cell-cluster microscopic creatures, such as prokaryotes, which includes bacteria and certain types of algae, and eukaryotes, which includes fungus and protists. The subfields of microbiology include bacteriology, virology, mycology, parasitology, and others. According to its definition, microbial physiology is the study of how microbial cell architecture, development, and metabolism work in living creatures. It includes the study of parasites, bacteria, fungus, and viruses. The investigation of microbial growth, metabolism, and cell structure is a component of the research of microbial cell functions. Functional genomics and metabolic engineering both benefit from understanding microbial physiology. Microbial physiology is a vast field that encompasses the study of tens of thousands of different microorganisms.

KEYWORDS:

Bacteria, Bacterial Growth,Microorganisms, Microbiology, Microbial Physiology.

INTRODUCTION

This book, Microbial Physiology, is divided into fourteen units that will help you comprehend the fundamentals of microbiology, bacterial growth and nutrition, growth of bacteria, phases of growth, growth kinetics, continuous culture, and synchronous culture, nutritional diversity in microorganisms, nutritional types, chemotrophism, CO2 assimilation, and reductive acetyl CoA pathway, as well as chemoheterotrophism, bacterial photosynthesis, and structure[1]–[3].The book uses the SIM format, which starts each unit with an introduction to the subject and ends with a list of the unit's objectives. For better comprehension, 'Check Your Progress' questions and answers are inserted throughout the text in a straightforward, organised format. For efficient recapitulation, each unit includes a list of "Key Terms," a "Summary," and a series of "Self Evaluation Questions and Activities".

Nutrition and Bacterial Growth

Microscopically small, single-celled creatures known as bacteria may survive in a variety of conditions. They may survive in the water, on land, and even in the human digestive system. Bacteria and humans have a complicated connection. Sometimes they assist us by aiding in digestion or curdling milk into yoghurt. At other times, they are harmful, spreading conditions like pneumonia and MRSA. On Earth, bacteria are among the most prevalent living forms. Every ecosystem contains them, and they are essential to daily existence. Bacteria, for instance, have an impact on what humans breathe, consume, and drink.

Due to their significance, it is preferred to research certain bacterial species in a lab setting. To do this, a single species of bacterium is cultivated in pure culture under carefully monitored circumstances. In pure culture, bacteria proliferate swiftly and rapidly increase in cell number. A "growth curve" is created by tracking the tempo of cell population expansion. This is crucial when trying to use or inoculate known quantities of the bacteria, such as when trying to boost plant growth, accelerate the breakdown of harmful chemical compounds, or industrially generate natural goods like antibiotics.One bacterial cell splits into two in the process of binary fusion, which is how bacteria multiply. The amount of time needed for a cell to divide is known as the mean generation time, sometimes referred to as the doubling time, which is the period of time needed for a cell to produce two times as many cells.You will learn about bacterial growth in this unit, including the phases of development, growth kinetics, and variables influencing growth.

Introduction to Bacteria In General

On Earth, bacteria are among the most prevalent living forms. Every ecosystem contains them, and they are essential to daily existence. In fact, a person's body contains more bacterial cells than mammalian cells, and bacteria may alter things like what they eat, drink, and breathe. Due to their significance, it is preferred to research certain bacterial species in a lab setting. To do this, a single species of bacterium is cultivated in pure culture under carefully monitored circumstances. In a short amount of time, bacteria in pure culture multiply rapidly and substantially expand in cell quantity. A "growth curve" is created by tracking the tempo of cell population expansion. This is crucial when trying to use or inoculate known quantities of the bacteria, such as when trying to boost plant growth, accelerate the breakdown of harmful chemical compounds, or industrially generate natural goods like antibiotics.The number of cells doubles as a consequence of every cell division.The rise is modest at low cell densities, but after a few generations, cell densities explode. 2n cells remain after n divisions.

Bacteria Growth

They are put in a flask where the nutrition supply and ambient parameters are regulated in order to comprehend and characterise the development of certain bacteria. The increase in numbers may be calculated as a function of time to produce a growth curve if the liquid medium provides all the nutrients necessary for development and the growth-friendly environmental conditions. A growth curve has a number of unique growth stages.

Growth Stages

There are four stages of development, which are called the lag phase, exponential or log phase, stationary phase, and death phase. Each stage is characterised by certain physiological changes

The Lag Phase

Bacteria do not quickly divide after being introduced onto new, fresh medium. It takes time for bacteria to adapt to a new habitat. The lag phase of bacteria is the period during which they are metabolically active but do not divide. The time frame in which there is no rise in the number of cells is known as the lag phase. Bacteria grow continually, reaching their greatest size at the conclusion of the lag phase. Microorganisms attempt to adapt at this phase to a new environment. The period of adaptation required for the creation of enzymes and co-enzymes for physiological activities is known as this phase. The physical environment around each cell needs time to change. The length of the lag phase varies depending on the

environment and the kind of bacteria. The length will be greater if the culture organism is taken from an old culture; but, if the culture is new, the duration will be short. Similar to this, if the culture medium differs from the previous culture, the duration will be prolonged since it takes bacteria some more time to adapt to the new medium. Bacteria are completely ready for cell division after the lag phase is over.

Exponential Phase vs Log Phase

The number of bacteria multiplies exponentially during this phase as they divide continually at a steady pace. All bacteria are now in this phase of balanced growth and fast cell division. Bacteria are at their lowest size during this phase due to fast cell division. In terms of their metabolic processes, cellular chemistry, and other physiological traits, the bacterial population is very consistent. Bacterial identification is often based on biochemical and physiological traits that emerge during the log phase of development. In the log phase, bacteria's generation time is often calculated. Not every bacterium in culture experiences this, however. The shortest generation time occurs during the log phase, and it is highly reliant on the growth components available in the medium. Depending on the kind of organism, the environment for development, and the density of the organisms, this phase lasts for many hours.

Stationary Phase

When this happens, there is no longer a net increase in the population of bacteria. A stagnant phase is what this is. By maintaining a balance between cell division and cell death throughout this phase, a stable bacterial population is maintained. There is no net change in the quantity of germs since some bacteria completely stop dividing their cells. Increased bacterial cell density, a decrease in medium nutrition, and a buildup of toxic secondary metabolic wastes all contribute to the stationary phase. Some bacteria produce enzymes and antibiotics during the stationary phase of their development, including penicillin, streptomycin, and others. As bacteria reach stationary phase in endospore-forming bacteria, sporulation takes place.

The Decline Phase or Death Phase

Bacteria numbers in this phase are continually and rapidly declining. The overall number of bacteria may stay constant throughout this period, but the number of viable bacteria declines. It just has the log phase's opposite. However, the rate of mortality is lower than the pace of growth. Many factors, including nutrient loss and the buildup of hazardous wastes, cause the death phase. Not all bacteria decompose at the same pace; spore-forming bacteria, for instance, exhibit both quicker and more durable decomposition rates.

Bacteria: Growth and Development Phases Kinetics

Batch culture, continuous culture, and synchronic culture are the three types of growth kinematics.The pace at which the total number of cells in a given system increases is referred to as the growth kinetics. The study of microbial growth kinetics, however, goes beyond the straightforward dynamic recording of biomass increase over time in a culture to include the extraction of parameters that allow for the quantitative formulation of general principles, the creation of mathematical models that allow for the description and prediction of microbial growth processes, and the establishment of a foundation for further experimentation. Because of this, growth kinetics is a crucial tool not only in the applied domains of industrial and environmental biotechnology but also in the basic sciences of microbial genetics, physiology, and ecology, including competition, selection, and evolution[4]–[6].

Growth Kinetics in Microbes

The ratios of dX/dt and dS/dt are almost zero during the lag period. To define crucial microbial kinetic parameters, it is feasible to monitor dX/dt and dS/dt values when the exponential growth phase starts:

Earnings Coefficient

Three kinds of microbial growth kinetic relationshipsmonod, first-order, and zero-order kineticswere developed using the yield coefficient and the specific growth rate.The growth pattern of a microbe in a fermenter is discussed in Microbial Growth Kinetics. When selecting the ideal batch time for product recovery, this element has particular importance. On the basis of a typical batch type fermentation, which contains the following phases: Lag Phase, Logarithmic/Exponential Phase, Static/Stationary Phase, Death/Decline Phase, a typical growth curve is described.The lag phase is when there is little to no growth in the fermenter. The requirement to adapt to the new environment is the reason why inoculum is injected into the medium.

Logarithmic/Exponential Phase: When the inoculum adapts to the environment, this phase shows a rapid increase in population as a result of the inoculum's quick division. It is also during this phase that we witness the fastest cellular growth rate and the quickest absorption of nutrients or substrate.

Static/Stationary Phase: During this phase, the number of microbes stops growing and there is balance between the number of cells that divide and the number that die. The loss of vital nutrients for development and the buildup of harmful byproducts are causes of growth cessation. Here, the production of products not related to growth is shown.

The microbe's last phase of its development cycle is known as the death or decline phase. The number of cells dividing will be less than the number of cells dying in this situation. The main causes of cell death are the buildup of harmful substances and the depletion of all nutrients in the medium.Fermentation systems used for batches are closed systems. At time t=0, microorganisms are added to the sterilised nutrient solution in the fermenter, and the incubation process is permitted to continue for an appropriate amount of time in a gaseous atmosphere and at a suitable temperature. Just oxygen, an anti-foam agent, an acid or base to regulate pH, and these are provided during the whole fermentation process. As a consequence of cellular metabolism, the composition of the medium, the concentration of biomass, and the concentration of metabolites all typically fluctuate continuously. Six typical stages of development are seen once bacteria are added to a sterile nutrient solution and grown there under physiological circumstances.

Nutrient intake contributes to growth. The first lag phase is a period in which there is no visible growth, but biochemical studies reveal metabolic turnover, suggesting that cells are in the process of adjusting to the environmental circumstances and that new growth will ultimately start. Then, when the inoculum starts to develop, there is a transitory acceleration phase that is swiftly followed by an exponential phase. When nutrients are in abundance, the environment is optimal, and growth inhibitors are not present, microbial growth occurs at the fastest pace conceivable for that particular organism during the exponential phase. In batch culture, however, exponential growth has a short shelf life. When nutritional circumstances change, growth rate declines, entering the deceleration phase. This is then followed by the stationary phase, during which overall growth is no longer possible due to nutrient depletion. After the growth rate has stopped, the cycle's last phase is called the death phase. Due to decreased metabolism and cell lysis, the majority of biotechnological batch procedures end

before this point. Batch cultures are the most common kind of microbial cultures used in laboratories.The following benefits and drawbacks are offered by batch culture systems:

Advantages

1. Since the growing phase is brief, there is little chance of contamination or cell mutation.
2. Less expensive to start up than continuous operations for the same bioreactor capacity.
3. More adaptability to various product/biological systems.
4. Greater raw material conversion rates as a consequence of a regulated development phase.

Disadvantages

1. Reduced levels of production since it takes time to fill, heat, sterilise, cool, empty, and clean the reactor.
2. a greater attention on equipment because of regular sterilising; a higher cost associated with getting numerous subcultures ready for inoculation.
3. Bacteria: Growth Phases and Growth Kinetics
4. Greater industrial hygiene hazards because of the possibility for contact with harmful microbes or toxins; higher expenses for personnel and/or process control for this non-stationary technique.
5. Typical Batch Culture Uses
6. Items that need the least amount of contamination or organism mutagenesis during production.
7. Processes that only create little quantities of a product.
8. Procedures where batch or semi-continuous product separation is sufficient; procedures that produce many products utilising a single reactor.

OngoingFermentation

An open system is set up for continuous fermentation. Continuous sterile nutrient solution addition to the bioreactor is accompanied by the removal of an equal volume of transformed nutrient solution containing microorganisms from the system. A turbidostat or a chemostat may be used in a homogeneously mixed bioreactor. Changing the concentration of one substrate in the chemostat regulates cell growth in the steady state. The rate of feed of nutrient solution is suitably controlled in the turbidostat to maintain continuous cell development by utilising turbidity to measure the biomass concentration.With a turbidostat, continuous cell concentration is maintained while a constant chemical environment is maintained in a chemostat. The growing chamber and a reservoir of sterile media are joined in a chemostat. After growth has begun, the reservoir constantly supplies new media. There is some form of overflow drain that keeps the fluid volume in the growing chamber constant. The pace at which new media is introduced into the growth chamber controls the rate of bacterial growth. The fresh medium always contains a finite quantity of an important nutrient, hence the pace at which fresh medium is added controls the rate of development. Hence, the chemostat relaxes the conditions that cause the stationary phase of the development cycle to begin, which are an insufficient supply of nutrients, the buildup of toxic compounds, and the accumulation of too many cells in the culture.Using continuous cultures instead of batch cultures has a number of significant benefits and drawbacks, some of which are listed below.

DISCUSSION

Advantages

1. Opportunities for system inquiry and analysis are expanded by continuous responses. The process outcomes may be benchmarked while the variables are kept constant, and the impact of even little changes to physical or chemical factors can then be assessed. It is possible to monitor changes in cell composition and metabolic activity by altering the growth-limiting nutrient. Along with giving a more realistic picture of kinetic constants, maintenance energy, and genuine growth yields, the continuous process' consistency.
2. Compared to batch culture, continuous culture offers a better level of control. Growth rates may be controlled and sustained for a long time. The concentration of biomass may be regulated by changing the dilution rate. Growth and the generation of secondary metabolites may both continue. Contrary to batch processes, where one organism typically outgrows another, steady state continuous culture allows for the maintenance of mixed cultures using chemostat cultures. • Chemostat-operated bioreactors can be used to improve selectivity for thermophiles, osmotolerant strains, or mutant organisms with rapid growth rates. Moreover, the medium composition may be adjusted utilising a pulse-and-shift technique, which injects nutrients right into the chemostat, to promote the growth of biomass and the production of products. A new steady state is created by adding the nutrient to the medium supply reservoir when changes are noticed.
3. The findings are more predictable and consistent because to the continuous culture's constant state, which results in a higher-quality final product.
4. When time-consuming processes like cleaning and sterilisation are eliminated, productivity per unit volume increases as well.
5. The capacity to automate the process makes it more cost-efficient and less susceptible to the effects of human mistake.

Disadvantages

1. It is difficult to oversee the production of certain non-growth-related items. Wall development and cell aggregation may also induce wash-out or hinder optimal steady-state growth, which is why the continuous process often necessitates feed-batch culturing and a continual food supply.
2. If a faster-growing strain overtakes the original product strain, it can become extinct over time.
3. The mixture's viscosity and heterogeneity may also make it challenging to sustain filamentous organisms.
4. Extended growing durations not only raise the danger of contamination but also need very consistent and dependable bioreactors, which might result in a greater initial outlay for better equipment. synchronised expansion.

A bacterial population grows synchronously when all of its members are physiologically similar and going through the same stage of the cell division cycle at the same time. Studying certain phases of the cell division cycle or their interactions is made easier by synchronous growth.It is challenging to understand the qualities throughout the cell division cycle using most bacterial cultures since the phases of growth and cell division cycle are entirely random. To solve this issue, microbiologists created synchronous culture methods to detect synchronous bacterial population increase.A synchronous culture is one in which all of the bacterial cells in the population are physiologically similar and are going through the same

stage of the cell cycle at the same time.By adjusting environmental factors, such as by repeatedly altering the temperature or by introducing new nutrients to cultures as soon as they reach the stationary phase, or by physically separating the cells using centrifugation or filtration, a synchronous culture may be produced.The Helmstetter-Cummings Method is the best and most popular way to create synchronous cultures. It involves filtering an unsynchronized bacterial culture via a cellulose nitrate membrane filter.

Bacterial cells that aren't strongly linked to the filter are washed away, leaving some cells behind. Now that the filter is upside down, new medium may pass through it.The filter is not weakly related with new bacterial cells generated by cell division that are washed into the effluent. As a result, the effluent contains only recently produced cells that are all in the same stage of their development and division cycle. Hence, the effluent stands for a synchronised culture.

Factors Affecting the Growth of Beetles

Similar to more sophisticated creatures, microorganisms need a range of resources from their surroundings in order to operate and achieve their two main objectives: provide adequate energy to control their activities and extract building blocks to repair or reproduce. Microorganisms not only survive on what they consume, but also in certain habitats. Even the quantity and distribution of materials in any given habitat may be crucial. These environments fluctuate as much as the organisms themselves do. With the use of this knowledge, researchers may cultivate microorganisms in the lab for testing.

Nutrients

All microbes need nourishment. The dietary needs of various bacteria vary. Many materials may serve as food sources, but predominantly proteins, lipids, and carbohydrates are used by the organisms to extract carbon and nitrogen. Such particles are sought for and absorbed by certain microbes. Others may carry out chemical interactions with nearby substances like carbon dioxide to get what they need, while yet others may use photosynthesis to create their own simple sugars in a manner akin to plants. The surrounding environment or other organic substances may provide the nitrogen needed to create proteins.Bacterial growth happens more quickly in culture medium that is rich in substances that encourage growth. Growth rate reduces as nutrient concentration rises. Bacterial growth rates rise up to a certain point with an increase in nutrition concentration before remaining constant regardless of additional nutrition.

Temperature

In general, bacteria may grow more readily up to a certain degree the higher the temperature. Both very high and extremely low temperatures interfere with the enzyme reactions necessary for bacteria to live, although different species of. They are often divided into three categories by scientists: psychrophiles, mesophiles, and thermophiles.

1. Psychrophiles like temperatures between 0 and 5 °C.
2. Mesophiles like temperatures between and degrees Celsius.
3. Thermophiles thrive at temperatures of at least degrees because they like the heat.

Bacterial growth rates increase as temperature rises steadily from its lowest point because temperature increases speed up metabolic processes. The growth rate reaches its maximum at a certain temperature, which is referred to as the ideal temperature. As the temperature is raised above the optimum level, the growth rate starts to decline but stops suddenly when it reaches the maximum temperature.Also, microorganisms like certain acidic properties in their

surroundings, or a particular pH level in the material or habitat in which they develop. Neutrophils, which favour a neutral pH level, make up the majority of microorganisms, including the majority of human diseases. Certain organisms like high pH values, but most typically, if the environment is too acidic, the organism's enzymes degrade.

For microorganisms to exchange resources between their cells and for their metabolic activities, water must be able to flow freely. All microorganisms need some amount of water, but some are able to thrive in low-moisture environments by preserving every drop of water they come across and by remaining in an area with plenty of moisture. Yet generally speaking, the presence of bacteria increases with wetness[7]–[9].

Elements in Play

Microorganisms often need the presence of certain airborne components in addition to water in order to manufacture the necessary nutrients. One such element is nitrogen, along with oxygen and carbon dioxide. Aerobic respiration needs oxygen, therefore obligatory aerobic bacteria must need oxygen to grow. Bacillus, for instance, or Mycobacterium. Oxygen may be dangerous or even fatal for obligate anaerobes. Facultative anaerobes, however, may withstand low oxygen concentrations. Capnophilic bacteria need carbon dioxide to survive. like Helicobacter pylori and Campylobacter.

Salt and Ions

For the synthesis of enzymes and proteins, all bacteria need metal ions such as K+, Ca++, Mg++, Fe++, Zn++, Cu++, Mn++, etc. The majority of bacteria are not salt-sensitive;however, they may withstand extremely low salt concentrations in medium. Certain halophilic bacteria, including Archaebacteria, need a lot of salt in their medium to survive[10]–[12].

1. The pace at which the quantity of distinct cellsor, more generally, the quantity of active biomasschanges in a certain system is referred to as the growth kinetics.
2. The microbe's death or decline phase marks the end of its development cycle. In this case, there will be more dead cells than there are dividing cells.
3. The depletion of all nutrients in the medium and the buildup of hazardous chemicals are the main causes of cell death during the death phase.
4. There are several methods for carrying out microbial culture operations. In industrial settings, three types of fermentation are used: batch, continuous, and fed batch.
5. Batch culture has two benefits:
6. Less chance of contamination or cell mutation due to the rapid development.
7. Reduced capital expenditure for the same bioreactor capacity as compared to continuous processes.
8. Batch culture has two drawbacks:
9. Reduced production levels caused by the time required to fill, heat, sterilise, cool, empty, and clean the reactor.
10. More attention being paid to instruments because of regular sterilising.
11. Synchronous growth makes it easier to investigate certain phases of the cell division cycle and how they relate to one another.
12. Nutrients, temperature, pH levels, water, elements present, ions, and salt are variables that impact microbial development.

CONCLUSION

The lag phase refers to the moment when bacteria are metabolically active but do not divide. The absence of a cell count rise during the lag phase is what distinguishes. The number of bacteria grows exponentially during the log phase as they divide continually at a constant pace. There are four stages of development, which are referred to as the lag phase, exponential or log phase, stationary phase, and dying phase. Each stage is accompanied by certain physiological changes. There is no longer a net increase in the bacterial population as a result of the bacteria's development. The term "stationary phase" refers to this. Maximum temperature is the greatest temperature that permits growth, while minimum temperature is the lowest temperature that does so. Below the bare minimum and above the maximum temperature, there is no growth. No development occurs below the minimal temperature because the cell membrane stiffens and hardens to carry nutrients into the cell. Bacterial growth stops at temperatures above the maximum because cellular proteins and enzymes become inactive.

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CHAPTER 2

EXPLORING THE ROLE OF MICROBIAL NUTRITION

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ABSTRACT:

Nutrients are substances that are obtained from the environment and are required for metabolism and development. The source, chemical form, and quantity of the key components that microorganisms or germs need differ greatly. Carbon, oxygen, hydrogen, phosphorus, and sulphur are a few examples of these vital nutrients. The two types of essential nutrients are macronutrients, which are required in vast quantities, and micronutrients, which are required in trace or minute quantities. Typically, macronutrients support the maintenance of cell metabolism and structure. Micronutrients support the upkeep of protein structure and enzyme functionality. Carbon and hydrogen atoms are present in organic substances in some combination. Defining inorganic nutrients as substances or simply molecules composed of substances other than carbon and hydrogen. Since there are two energy sources accessible to microbes, there are two different sorts of them: phototrophs, which get their energy from sunlight, and chemotrophs, which get their energy by oxidising either organic or inorganic chemical substances

KEYWORDS:

Microbial Nutrition, Bacterial Growth,Microorganisms, Hydrogen Atoms.

INTRODUCTION

You will learn about the variety of nutrition found in microorganisms, as well as about the forms of nutrition found in them, including autotrophy, heterotrophy, chemotrophy, phototrophy, lithotrophy, and organotrophy, as well as the importance of major and minor components to nutrition[1]–[3].

Nutritional Diversity

Organisms need a source of nutrients or raw materials in order to generate energy and build new cell components. Ingredients utilised in biosynthesis and energy generation are called nutrients.

Needs for Nutrients

According to microbial cell composition, just a few principal components make approximately % of the dry weight of a cell. Phosphorus, potassium, calcium, magnesium, iron, hydrogen, nitrogen, and sulphur. Macro- or macro-nutrients Microorganisms need them in rather high quantities. The elements that make up carbohydrates, lipids, proteins, and nucleic acids are carbon, oxygen, hydrogen nitrogen, sulphurs, and phosphorus. The cell has cations of the remaining four macro elements.K+ - Several enzymes, particularly those involved in protein synthesis, need K+ to function.

Bacterial endospores' ability to withstand heat is aided by Ca2+. Dipicolinic acid and calcium are present in % of the spore.Mg2+ - This mineral stabilises cell membranes and ribosomes as well as acting as a cofactor for several enzymes.Fe2+ and Fe2+ - Components of cytochromes and cofactors for enzymes and proteins that transport electrons.

Dietary supplements, or trace elements

Manganese, zinc, cobalt, molybdenum, nickel, and copper are among them. They are often found as cofactors and parts of enzymes, where they support the catalysis of processes and preservation of protein structure.When the regulatory and catalytic subunits of E. coli aspartate carbomoyltransferase are associated, Zn2+, which is present at the active site of several enzymes, is also implicated.The transfer of phosphate groups is catalysed by several enzymes with the help of Mn2+.

Mo2+ - Necessary for fixing nitrogen. Co2+ is a subunit of vitamin B

In addition to macro and micronutrients, certain microbes could have unique needs that reflect the unique aspects of their morphology or habitat. To build their stunning silica cell walls, diatoms need silicic acid. High sodium ion concentrations are required for bacteria to flourish in salty lakes and seas. All of the aforementioned nutrients must be present in a balanced ratio for microorganisms to develop properly [4]–[6].

DISCUSSION

For Carbon, Hydrogen, and Oxygen, There Are Needs

All organic compounds need carbon for the skeleton or backbone, and molecules acting as suppliers of carbon often also provide oxygen and hydrogen atoms. CO2 is a crucial carbon source that does not produce energy or hydrogen. Autotrophs - are organisms that can utilise CO2 as their only or main source of carbon. Many bacteria are phototrophic, meaning they utilise light to synthesise most of their energy. Certain autotrophs use the oxidation of inorganic substances to produce energy. Organisms classified as heterotrophs utilise reduced, already-formed organic molecules as carbon sources. For instance, the glycolytic cycle releases energy in the form of ATP and NADH while simultaneously producing the carbon skeleton needed for biosynthesis. Amyl alcohol, paraffin, and even rubber may all be broken down by actinomycetes. Almost 0 distinct carbon compounds are available for utilisation by Burkholderia cepacia. Even somewhat difficult-to-digest compounds created by humans, such pesticides, may be broken down by certain bacteria. In the presence of a growth-promoting nutrient that is metabolised concurrently, indigestible molecules may be oxidised and destroyed, a process known as co-metabolism. Other microbes may then utilise the breakdown products as nutrition [7]–[9].

Microorganisms of the Nutritional Types

For growth, all organisms need sources of energy and electrons in addition to carbon, hydrogen, and oxygen.

Carbon sources

1. Autotrophs - CO sole or principal biosynthetic carbon source
2. Heterotrophs - Reduced, preformed organic molecules from other organisms.
3. Energy sources
4. Phototrophs – Use light as their energy source.
5. Chemotrophs – Obtain energy from the oxidation of chemical compounds
6. Electron sources

7. Lithotrophs – Use reduced inorganic substances as their electron source.
8. Organotrophs – Extract electrons from organic compounds.
9. Four major nutritional classes based on their primary sources of carbon, energy and electrons are:
10. Photolithotrophic Autotrophs or Photoautotrophs or Photolithoautotrophs
11. Source of energy – Light energy
12. Source of electrons – Inorganic hydrogen/ electron
13. Carbon source - CO
14. Example: Algae, purple and green sulfur bacteria and cyanobacteria.
15. Photoorganotrophic Heterotrophy or Photoorganoheterotrophs
16. Source of energy – Light energy
17. Source of electrons – Organic hydrogen/ electron
18. Carbon source – Organic carbon sources
19. Example: Purple and green non-sulfur bacteria
20. Chemolithotrophic autotrophs or chemolithoautotrophs
21. Source of energy – Chemical energy source
22. Source of electrons – Inorganic hydrogen/ electron donor
23. Carbon source - CO
24. Example: Sulfur-oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron-oxidizing bacteria.
25. Chemoorganotrophic Heterotrophs or Chemoorganoheterotrophs
26. Source of energy – Chemical energy source
27. Source of electrons – Inorganic hydrogen/ electron donor
28. Carbon source – Organic carbon source
29. Example: Protozoan, fungi, most non-photosynthetic bacteria

The most common nutritional types are photolithoautotrophs and chemoorganoheterotrophs. Bacteria Beggiatoa rely on inorganic energy sources and organic carbon sources. These microbes are sometimes called mixotrophic because theycombine chemolithoautotrophic and heterotrophic metabolic processes.

Microbial Nutrition

Requirement for Nitrogen, Phosphorus and Sulfur

It is necessary to synthesise nitrogen in order to create amino acids, purines, pyramidines, certain carbohydrates, lipids, enzyme cofactors, and other substances. The majority of phototrophs and a few non-phototrophs convert nitrate to ammonia and assimilate the ammonia by nitrate reduction. Many Cyanobacteria and Rhizobium, among other bacteria, employ nitrogenase systems to reduce and absorb atmospheric nitrogen. Phosphorus is a component of nucleic acids, phospholipids, ATP, a variety of cofactors, certain proteins, and other cell components. All microorganisms quickly use inorganic phosphate as their supply of phosphorous. E. E. coli uses both inorganic and organic phosphate. Organophosphates like hexose 6-phosphate may be directly taken up by transport proteins. Other organophosphates are often hydrolyzed by alkaline phosphatase in the periplasm to produce inorganic phosphate, which is then transported through the plasma membrane. When inorganic phosphate is available outside of the bacterium, it enters through the outer membrane through a porin protein channel. Sulfur may be used to create the amino acids cysteine and methionine, as well as certain types of carbohydrates, biotin, and thiamine. Some of them need sulphur in a reduced state, like cysteine, but the bulk get their sulphur from sulphate and reduce it by absorption.

Image Factor

The enzymes and metabolic pathways needed to synthesise each component of a cell are found in several microorganisms. Many organisms need organic molecules since they cannot produce them on their own yet are crucial cell components or precursors to such components. Growth factors are the name given to these compounds.The three main categories of growth factors are:

1. Amino acids are necessary for the production of proteins.
2. Purines and Pyramidines - for the production of nucleic acids
3. Just extremely little quantities of vitamins, which are chemical compounds that often make up all or a portion of the cofactors for enzymes, are necessary for development.

Quantitative growth response tests for a range of compounds are now achievable because to knowledge of the particular growth factor needs of numerous bacteria.Its usage in industry is a result of the discovery that several microbes can manufacture significant amounts of vitamins. Industrial fermentations are used to create certain vitamins that are both fat- and water-soluble.

a. Riboflavin – Clostridium, Candida, Ashbya, Eremothecium
b. Coenzyme A – Brevibacterium
c. Vitamin B – Streptomyces, Propionibacterium, Pseudomonas
d. Vitamin C – Gluconobacter, Erwinia, Corynebacterium
e. â- Carotene – Dunaliella
f. Vitamin D – Saccharomyces

1) Microbial cell composition shows that % of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.
2) Some of the micro-nutrients are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.
3) Some of the macro-nutrients are carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements exist in the cell as cations.
4) Carbon is needed for the skeleton or backbone of all organic molecules and molecules servingas carbon sources normallyalso contribute both oxygen and hydrogen atoms.
5) Organisms that uses carbon as a source are autotrophs as CO2 sole or principal biosynthetic carbon source and heterotrophs as educed, preformed organic molecules from other organisms.
6) Organisms that uses energy as a source are phototrophs use light as their energysource and chemotrophs obtain energyfrom the oxidation of chemical compounds
7) Organisms that uses electron as a source are lithotrophs that uses reduced inorganic substances as their electron source and organotrophs use extract electrons from organic compounds.
8) Four major nutritional classes based on their primary sources of carbon, energy and electrons are -
   i. Photolithotrophic autotrophs or photoautotrophs or photolithoautotrophs
   ii. Photoorganotrophic heterotrophy or photoorganoheterotrophs
   iii. Chemolithotrophic autotrophs or chemolithoautotrophs
   iv. Chemoorganotrophic heterotrophs or chemoorganoheterotrophs.

9. Nitrogen is needed for the synthesis of amino acids, purines, pyramidines, some carbohydrates and lipids, enzyme cofactors and other substances.

i. Most phototrophs and many non-photosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction.
ii. Phosphorous is present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components.
iii. E. coli can use both organic and inorganic phosphate.
iv. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel.
v. Nutrients are materials that are acquired from the environment and are used for growth and metabolism.
vi. Microorganisms or microbes vary significantly in terms of the source, chemical form, and amount of essential elements theyneed. Some examples of these essential nutrients are carbon, oxygen, hydrogen, phosphorus, and sulfur.
vii. There are two categories of essential nutrients: macro-nutrients which are needed in large amounts and micro-nutrients which are needed in trace or small amounts.
viii. Macro-nutrients usually help maintain the cell structure and metabolism.
ix. Micro-nutrients help enzyme function and maintain protein structure.
x. Organic nutrients contain some combination of carbon and hydrogen atoms.
xi. Inorganic nutrients are elements or simply molecules that are made of elements other than carbon and hydrogen.
xii. There are two sources of energy available to microorganisms, and based on this they are of two types, i.e., phototrophs in which energy for growth is derived from sunlight and chemotrophs in which energy for growth is derived from the oxidation of either organic or inorganic chemical compounds.
xiii. To obtain energy and construct new cellular components, organisms, must have a supply of raw materials or nutrients. Nutrients – are substances used in biosynthesis and energy production.
xiv. According to microbial cell composition, only a few key substances account for % of the dry weight of a cell. Iron, phosphorus, potassium, calcium, magnesium, hydrogen, nitrogen, sulphur, and carbon.
xv. Microorganisms need rather significant levels of macronutrients or macro components. Carbohydrates, lipids, proteins, and nucleic acids are made up of carbon, oxygen, hydrogen nitrogen, sulphurs, and phosphorus. The cell contains cations of the other four macro elements.
xvi. The trace elements manganese, zinc, cobalt, molybdenum, nickel, and copper are examples of micronutrients. They are often cofactors and components of enzymes, and they support the upkeep of protein structure as well as the catalysis of processes. In addition to macro and micronutrients, certain microbes may have unique needs that reflect the unique aspects of their morphology or habitat.
xvii. Silicic acid is required by diatoms to build their stunning silica cell walls. High sodium ion concentrations are necessary for the growth of bacteria in salty lakes and seas.

Microorganisms need a well-balanced mixture of all the aforementioned nutrients for optimal growth. All organic compounds need carbon for their skeleton or backbone, and oxygen and hydrogen atoms are often present in molecules that serve as carbon suppliers. The main source of carbon that does not generate energy or hydrogen is CO2. When a nutrient that promotes development is also being metabolised simultaneously, a process known as co-metabolism allows for the oxidation and destruction of indigestible molecules.Bacterial

Multiplication and Growth By using binary fission, bacteria reproduce. After a bacterial cell reaches a certain size, it divides into two daughter cells. Nuclear division is followed by cell division. The length of time required for a bacterium to divide into two daughter cells under ideal conditions is known as the generation time or population doubling time. Escherichia coli and many other essential bacteria for medicine may be produced in as little as minutes. Some bacteria grow very slowly. The tubercle bacilli's production process takes around hours.

With lepra bacteria, it might persist for up to days. After a few cell divisions, the multiplication of bacteria grown in liquid medium is terminated due to nutritional exhaustion or the accumulation of dangerous chemicals. It is a batch-based culture. It is possible to maintain a continuous culture of bacteria for scientific or industrial purposes by utilising specialised machinery for resupplying nutrients and removing bacterial cells [10], [11]. Bacterial growth in host tissues may occur either in batch or continuous cultures, depending on the situation. The host's defence mechanisms must still be overcome by the bacterium even if the source of nutrients may never run out. To determine how many viable cells are present, dilution or plating procedures are performed. The dilution technique involves serially diluting the sample whose cell count has to be calculated. Unit quantities are diluted until, when injected into the proper liquid media, they no longer support growth. Each dilution is injected into a tube filled with the appropriate liquid medium. The viable count is determined statistically based on the number of tubes demonstrating growth. This method is used to calculate the "presumptive coliform count" in drinking water, despite being erroneous [12], [13].

CONCLUSION

The presumptive coliform count is a method for figuring how how contaminated the water is. Using the plating approach, the appropriate dilutions are inoculated on solid medium, either on the surface of plates or as pour plates. The number of colonies that appear after incubation may be used to determine the viable count. The method described by Miles and Misra, in which colony counts are obtained after multiple dilutions are dropped on the surface of dried plates, is one that is often used. Bacterial colonies grow on solid media. Each colony is made up of cells that are ancestors of another cell. In a liquid media, growth is diffuse. Two types of bacterial growth may be distinguished: an increase in cell size and an increase in cell number. Population increase may be studied using bacterial counts. Total and viable bacterial counts may be separated into two groups. The total count represents the total number of cells in the sample, whether or whether they are alive. There are several methods that may be utilised, including direct counting in counting chambers under a microscope. The number of cells that are alive and capable of proliferating is measured by the viable count.

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CHAPTER 3

BACTERIAL PHOTOSYNTHESIS, NITROGEN METABOLISM AND STRESS RESPONSE

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ABSTRACT:

The existence of photosynthetic bacteria predates the time when the Earth's atmosphere could support human life. Yet, scientists have only just started to solve the puzzle of how these microscopic creatures carry out the processes of photosynthesis. Scientists are actively researching photosynthetic bacteria and learning important information about how they photosynthesize and how they have developed, despite the fact that they have not yet been able to fit all the pieces of the jigsaw together for these organisms. They even think that these microbes may have played a significant role in how the globe developed and may hold the key to life in previously thought to be inhospitable environments, such as Polar Regions like Antarctica and even other planets. Since they do not employ water as an electron donor, bacteria that have bacteriochlorophyll do not create oxygen. Known as anoxygenic photosynthesis, this process. Similar to plants, cyanobacteria use water as an electron donor during photosynthesis. This process, referred to as oxygenic photosynthesis, produces oxygen. The photosynthetic cyanobacteria, purple and green bacteria, and "halobacteria" are prokaryotes that can transform light energy into chemical energy.

KEYWORDS:

Bacterial Photosynthesis, Microorganisms, Creatures, Photosynthesis.

INTRODUCTION

As the name implies, these microorganisms are unique varieties of bacteria that have reaction centres and pigments that can absorb light, enabling them to transform light energy into chemical energy. Chlorophyll is found in cyanobacteria whereas bacteriochlorophyll is found in other types of bacteria. While having a similar structure to chlorophyll, bacteriochlorophyll absorbs light with a longer wavelength. The most prevalent type of bacteriochlorophyll is bacteriochlorophyll a, however there are other forms in b, c, d, e, f, and g. The extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to convert light energy into ATP. The cyanobacteria conduct plant photosynthesis, known as oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; and the purple and green algae.You will learn about the structure of photosynthetic pigments, including chlorophylls, bacteriochlorophyll, carotenoids, and phycobilins, as well as the main forms of microbial photosynthesis, oxygenic and anoxygenic [1]–[3].

The General Introduction to Photosynthesis

Although though the process of photosynthesis is most often associated with plants and algae, studies of photosynthetic bacteria have contributed significantly to our knowledge of the molecular foundation for light energy collection and photochemical energy transduction.

Photosynthesis, or the conversion of light energy into chemical energy in the form of ATP, is the utilisation of light as a source of energy for growth. The photosynthetic cyanobacteria, purple and green bacteria, and "halobacteria" are prokaryotes that have the ability to transform light energy into chemical energy. The extreme halophilic archaea use a type of non-photosynthetic photophosphorylation mediated by a pigment, bacteriorhodopsin, to convert light energy into ATP. The cyanobacteria conduct plant photosynthesis, known as oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; and the purple and green algae.

Bacterial Photosynthesis Types

There are five photosynthetic groups in the bacterial domain.

Oxygenic Photosynthesis: Cyanobacteria and prochlorophytes carry out this process, which causes the release of molecular oxygen and the removal of carbon dioxide from the atmosphere.

1. Takes place in lamellae that include thylakoids with pigments that capture light energy, including chlorophyll a/b and phycobilisomes.
2. Makes use of two photosystems

To create ATP, PS II produces a proton-motive force.

Photosynthetic Pigments' Structure

Many phototrophic organisms include a variety of different pigments.

Chlorophyll

All organisms that engage in photosynthetic growth use chlorophyll as their main pigment for light absorption. A tetrapyrrole called chlorophyll has magnesium in the porphyrin ring's middle. It connects with the photosynthetic membrane because to a lengthy hydrophobic side chain. Chlorophyll is present in cyanobacteria just as it is in plants and algae. Bacteriochlorophylls, often known as purple and green bacteria's chlorophylls, vary chemically from chlorophyll due to their substituent side chains. Their light absorption spectra demonstrate this. Bacterial chlorophylls absorb light from 0- nm in the far red area of the spectrum; chlorophyll absorbs light in two sections of the spectrum, one about 0 nm and the other between 0 and 0 nm.

Bacteriochlorophyll

In purple photosynthetic bacteria, bacteriochlorophyll, a structurally related magnesium porphyrin with a more saturated tetrapyrrole ring, serves as the major light-harvesting pigment instead of chlorophyll. Because of this, BChl absorbs in the near infrared at far longer wavelengths than chlorophyll; the absorbance spectrum is determined by the specifics of the macrocycle's conjugated electron system. A variety of carotenoids, which are the primary pigments in the visible area of the spectrum and give purple bacteria their purple colour, also participate in light harvesting. The absorbance spectrum of photosynthetic membranes made from Rhodobacter sphaeroides. BChl is responsible for the significant near-infrared absorbance as well as the band about 0 nm, whereas carotenoid is primarily responsible for the absorbance between 0 and 0 nm. The near-UV region between 0 and 0 nm is where the so-called Soret bands of BChl are found [4]–[6].

Carotenoids are constantly linked to the machinery used for photosynthetic processes. They serve as secondary light-harvesting pigments, absorbing light in the 0–0 nm range of the

blue–green spectrum. Carotenoids use wave lengths of light that chlorophyll misses to transmit energy to chlorophyll with a near 0% efficiency. Carotenoids also play a crucial role in preventing photooxidative damage to the photosynthetic machinery. Long hydrocarbon side chains in a conjugated double bond structure are present in carotenoids. The potent oxygen radical singlet oxygen, which is usually created in interactions between chlorophyll and O2, is "quenched" by carotenoids. Staphylococcus aureus is one example of a non-photosynthetic bacterial pathogen that produces carotenoids, which shield the cells from fatal singlet oxygen oxidations in phagocytes.

Phycobilins

The primary light-harvesting pigments in cyanobacteria are phytobiliproteins. They can also be found in a few types of algae. They may be red or blue and absorb light between 0 and 0 nm in the centre of the spectrum. Phycobiliproteins are made up of proteins that have linear tetrapyrrole bonded to them covalently. They are housed in phycobilisomes, granules that are intimately related to the photosynthetic machinery. They may effectively transport light energy to chlorophyll in the reaction centre because to their tight relationship with it.Cyclic photophosphorylation is a process that can be carried out by any phototrophic bacteria. Photosystem I is the name given to this common cyclic photophosphorylation process. Just Photosystem I is used in bacterial photosynthesis, but more advanced cyanobacteria, algae, and plants have a second light-harvesting system called Photosystem II. When electrons are taken out of Photosystem I for CO2 fixation, Photosystem II is utilised to diminish Photosystem I. PSII creates O2 by transferring electrons from H2O.

The Calvin cycle and the utilisation of RUBP carboxylase are the most typical mechanisms used by autotrophs to fix CO2. It is true that RUBP carboxylase is thought to be the most common enzyme on earth. The only eukaryote that uses this method of autotrophic CO2 fixation is the purple bacterium and all cyanobacteria. Bacteria that are lithoautotrophs also employ this route. Nevertheless, RUBP carboxylase is not present in green bacteria, methanogens, or a few isolated species of prokaryotes, which have alternate methods for autotrophic CO2 fixation.Ribulose bisphosphate and CO2 are co-substrates for RUBP carboxylase. By adding CO2 to the RUBP, which is then instantly split into two molecules of 3-phosphoglyceric acid, the CO2 is "fixed" in a complex process. One of the PGA molecules' - COO group eventually receives the fixed CO2. Indeed, the Calvin cycle is started by this reaction.

Photosynthesis in Bacteria

The Calvin cycle is concerned with the regeneration of RUBP, the substrate that powers the cycle, as well as the conversion of PGA into glycolysis intermediates that may be utilised for biosynthesis. After the first fixation of CO2, processes that are effectively the opposite of the oxidative Embden-Meyerhof pathway decrease and combine 2 PGA to create hexose-phosphate. Pentose-phosphate is created from the hexose phosphate and is then phosphorylated to create RUBP once again. The Calvin cycle plays a crucial role in providing the organic precursors needed for the production of cell components. Calvin cycle intermediates must be continuously removed in order to produce cell material. The Calvin cycle is an anabolic process in this sense. In order to fix CO2 to the same level as glucose, ATP and NADPH2 are needed.

The majority of phototrophic prokaryotes can fix CO2 as their only source of carbon for growth, making them autotrophs. Energy and electrons are needed to raise CO2 to the level of cell material, just as the oxidation of organic material produces CO2, electrons, and energy.

Energy for the nighttime CO2 fixation processes is produced by the light reactions in the form of ATP. Reductant is also required for dark reactions. The supply of electrons is often associated in some manner with the light processes. Anoxygenic processes like bacterial photosynthesis take place. Purple and green bacteria never make O2 during photosynthesis because H2O is never an external electron source for bacteria. Moreover, O2 often inhibits bacterial photosynthesis, which occurs in microaerophilic and anaerobic conditions. Bacterial chlorophylls don't have to compete with oxygenic phototrophs for light since they utilise light at longer wave lengths than those used in plant photosynthesis. Bacteria lack a second photosystem and solely employ cyclic photophosphorylation to synthesise ATP.

Energy Transduction in the Photosynthetic Bacteria

The heliobacteria, cyanobacteria, green sulphur bacteria, purple phototrophic bacteria, and green filamentous bacteria are the types of bacteria that employ chlorophyll-like molecules to harness sunlight as a source of energy. All except the last of them are classified as anoxygenic photosynthetic bacteria because they convert light energy into a physiologically usable form without producing oxygen via the oxidation of water. For a number of reasons, including metabolic adaptability, genetic accessibility, and the mostly straightforward and modular design of their photosynthetic machinery, the purple phototrophic bacteria are the group that has been the subject of the most research.In general, all organisms that have chlorophyll or bacteriochlorophyll use the same method to harness sun energy.

1. In the light-harvesting or "antenna" section of the photosystem, pigment molecules absorb light energy and temporarily store it in an excited electronic state.

2. The reaction centre portion of the photosystem, a pigment-protein complex encased in a charge-impermeable lipid bilayer membrane, receives excited state energy.

3. A photochemical reaction that separates a positive and negative charge across the breadth of the membrane is triggered by the arrival of the excited state energy at a specific bacteriochlorophyll, or pair of BChls, at the reaction centre.

4. Charge separation starts a chain of electron transfer events that are connected to the movement of protons across the membrane, creating an electrochemical proton gradient (also known as a proton motive force) that may be utilised to drive processes like the creation of ATP.

In purple phototrophic bacteria, the simplest photosynthetic unit consists of a reaction centre encircled by a light-harvesting complex termed LH1. Together, they make up the so-called RC-LH1 complex, which works with another membrane-embedded electron transport protein, the cytochrome bc1 complex, to transform light energy into a PMF. One or more kinds of peripheral antenna complexes, known as LH2, LH3, and so on, are added to certain species to increase their capacity for light collecting.

Peripheral Antenna - The light-harvesting pigment-proteins of purple bacteria have a cylindrical construction, as shown by X-ray crystallography and other structural approaches. The peripheral LH2 complex in Rhodopseudomonas acidophila is made up of nine copies of each of two short polypeptides called and ß, which each contain a single membrane-spanning -helix. The light-harvesting BChls and carotenoids are sandwiched between two concentric protein cylinders formed by these and the ß polypeptides in the membrane.Perspectives may be both parallel and perpendicular to the membrane's plane. Concentric cylinders of nine, and nine ß polypeptides make up the protein scaffold. The nine B0 BChls are shown as green sticks, whereas the B0 BChls are displayed as alternating red and orange spheres. The B0 and

B0 BChls' macrocycles are organised parallel to and perpendicular to the membrane's plane, respectively.

When aligned roughly on the membrane's plane, the BChls in LH2 create two rings. The first of them consists of BChls, one for each and ß polypeptide, and is arranged such that each BChl's macrocycle is about perpendicular to the membrane's plane. The absorbance band at 0 nm is clearly seen in these "B0" BChls. The second ring has nine "B0" BChls that are positioned with their macrocycles parallel to the membrane's plane. This arrangement results in an 0 nm absorbance band that is clearly visible. Furthermore, the LH2 complex includes light-harvesting carotenoid. Several species' peripheral LH complexes exhibit variations on this theme; for instance, Rhodospirillum molischianum's LH2 has eight pairs of and ß polypeptides. The 0 and 0 nm absorbance bands of the LH2 from Rba. sphaeroides are visible in the spectra, and it is remarkably comparable to that from Rps. acidophila.

Antenna core the most comprehensive data currently available comes from a 4.8 structure for the RC-LH1 complex from Rps. Palustris since a high resolution X-ray crystal structure for an RC-LH1 complex has not yet been disclosed. This displays a cylindrical LH1 with an approximately oval cross section in the membrane plane around a central RC. The LH1 pigment protein is made up of two polypeptides also known as and ß, and it has a similar overall structure to the LH2 pigment protein. Each polypeptide contains a single membrane-spanning -helix, and between the protein cylinders is a ring of BChls. To accommodate the RC in the middle vestibule, which has pairs of and ß polypeptides in Rps. Palustris, LH1 is bigger than LH2. Carotenoids and a single kind of "B5" BChl are found in LH1, which is organised into rings with each BChl macrocycle oriented perpendicular to the membrane. The Rba. sphaeroides LH1 complex's 5 nm absorbance band may be recognised as a shoulder in the spectra.

Perspectives may be both parallel and perpendicular to the membrane's plane. Concentric cylinders of and ß polypeptides, as well as one W polypeptide, make up the protein scaffold. The B5 BChls are shown as spheres with their macrocycles organised perpendicular to the membrane's plane, alternately in red and orange. For polypeptides and cofactors, the central RC is shown as ribbons and spheres, respectively. PDB file 1PYH was used to build the figure.In Rps. Palustris, a second membrane-spanning polypeptide fills in the space left by the LH1 pigment-incomplete protein's encirclement of the central RC. The purpose of this component is unknown, however it has been hypothesised that it functions in a manner similar to the PufX polypeptide, a small component of the Rhodobacter sphaeroides RC-LH1 complex that is crucial for effective quinone exchange between the RC and the bc1.

Response Center - The antenna's carotenoids and BChls serve to provide the RC with energy in the excited state. A full description of the structure and mechanism of the purple bacterial RC is provided in the associated PSO module by Yocum on RCs. Below, for completeness, is a short overview of the well-researched Rba. sphaeroides complex. The nearly related RC from Blastochloris viridis is described in the Yocum module.Three polypeptides, ten cofactors, two bacteriopheophytins, four BChls, two ubiquinones, a carotenoid, and a non-heme iron are all included in the Rba. sphaeroides RC. Inside a protein framework created by the L- and M-polypeptides, the BChl, BPhe, and quinone cofactors are organised in two roughly symmetrical membrane-spanning branches. The reduction of a quinone occurs at the so-called QB site, which is located close to the cytoplasmic side of the membrane, as a result of a membrane-spanning four-step electron transfer triggered by the arrival of excitation energy at a "special pair" of 0 nm-absorbing BChls at the periplasmic end of the RC. The L and M polypeptides, which are mostly intra-membrane, surround the cofactors. The H polypeptide contains a single anchoring -helix and an extra-membrane domain. The atoms of

Mg and Fe are shown as purple or brown spheres, respectively, while the cofactors are represented as sticks. For clarity, the BChl, BPhe, and quinone cofactors' hydrocarbon side chains have been cut off. The colour of a carbon atom is P0. BPhes are pink, quinones are cyan, BChl dimer are yellow, monomeric BChls are green, carotenoid is teal. Nitrogens are seen in blue and red, respectively, together with oxygen. Along an axis of two-fold pseudo-symmetry that links P0 with the Fe atom, the BChl, BPhe, and quinone cofactors are organised in two membrane-spanning branches. The path of electron transfer is shown by a black arrow, the location of H+ uptake by a blue arrow, and the Q/QH2 association and disassociation, as well as the transport of electrons to P0+ via cyt c+, are indicated by dashed green arrows. PDB file 2BOZ was used to create the figure.

In a cyclic proton motive electron transfer system with a partner ubiquinol:cytochrome c2 oxidoreductase, the bc1, the RC functions as a light-powered cytochrome c2:ubiquinone oxidoreductase. This protein catalyses a bifurcated electron transfer in which one electron removed from ubiquinol is used to reduce cyt c+ and the second electron is used to reduce quinone on the opposite side of the membrane. It is similar to the mitochondrial protein bc1 and the b6f complex of oxygenic phototrophs. The Rba. sphaeroides bc1 protein's X-ray crystal structure has been established, and like other bc1 complexes, this protein is dimeric. A cyt b, which consists of a membrane-spanning helix bundle encasing two hemes known as bL and bH, is in the centre of the bc1 protein. They join an ubiquinol oxidase site close to the periplasmic side of the membrane with a quinone reductase site close to the periplasmic side to generate a membrane-spanning electron transfer chain. Moreover, the bc1 comprises a Rieske iron-sulfur protein with a 2Fe-2S centre and a cyt c1, both of which have membrane-spanning domains on the periplasmic side. Each Rieske protein forms a dimer with the extra-membrane domain joining the second monomer and the membrane-spanning -helix joining the first.

Mechanism of the Photosystem

A wide variety of light energy may be captured by the purple bacterial photosystem. A carotenoid pigment of an antenna complex transfers energy to a nearby BChl after photon absorption changes the absorbing pigment's electronic state to a singlet excited state. The Soret band in the blue/near-UV area, the Qx band in the red region, and the Qy band in the near-infrared are the three main absorbance bands of the BChls, as was previously mentioned. Internal conversion transforms the energy absorbed in the Soret or Qx regions into the lowest-energy Qy excited state.The arrangement of the Qy excited state energies of the LH2 and LH1 BChls ensures that energy is directed into the RC. Energy received by the B0 BChls inside a single LH2 protein is transferred to the ring of lower energy B0 BChls. The energy is subsequently transferred to a nearby LH2 or to the ring of LH1 B5 BChls that surrounds the RC. The P0 dimer of BChls in the RC is transferred excited state energy in the last step, resulting in photochemical charge separation. Hence, having red-shifted BChls closest to the RC allows excited state energy to funnel into the RC.

Green represents B0 BChls of LH1, red and orange represent B0 BChls of LH2 and B5 BChls of LH1, and yellow represents P0 BChls of the RC. For the energy transfer events shown by arrows, lifetimes in picoseconds are provided. Antenna carotenoids absorb energy, which is then transferred to nearby BChls. Femtosecond energy migration within the B0 or B5 pigment rings is indicated by double-headed arrows.Light harvesting complexes must be closely packed within the membrane because the rate of energy transfer decreases with the sixth power of the distance between the donor and acceptor. This will help to ensure that excited state energy is transferred to the RC efficiently with the least amount of emission losses. Since BChl has an excited state lifetime of only a few nanoseconds, energy absorbed

by antenna pigments must be transferred to the RC over a period of several tens to hundreds of picoseconds. On time scales of a few ps, energy transfer between weakly coupled BChls in adjacent LH complexes occurs and, along with B0B0 energy transfer, is explained by the Förster mechanism. The excitation is delocalized over the entire B0 and B5 rings, where the BChls are strongly coupled, with extremely fast fs "hopping times" between adjacent BChls. This process is better understood using an exciton approach involving delocalized electronic transitions.

The process of moving the excited state from the B5 BChls to the P0 BChl dimer is the slowest and takes between and ps because of the relatively great distance involved. This configuration, in which the protein scaffold creates an exclusion zone separating the charge-separating Chls or BChls of the RC from the light-harvesting Chls or BChls of the antenna, is a feature of both PS1 and PS2 of oxygenic photosynthesis. This architecture appears to serve two main purposes: to prevent unwanted electron transfer reactions between RC and antenna BChls from interfering with the efficiency of membrane-spanning charge separation, and to ensure that unproductive back transfer of excitation energy is slow compared to productive charge separation.The relevant Yocum module on RCs goes into great detail about the photochemical charge separation process. A list of the reactions the Rba. sphaeroides RC catalysed. P0 becomes an effective reductant with enough energy from the excited state to give an electron to the nearby BA BChl. This electron is subsequently passed in three steps to the quinone at the QB site near the cytoplasmic side of the membrane, the photooxidised P0 located near the periplasmic face of the membrane being re-reduced by a water-soluble c-type cytochrome.

A second light-induced charge separation results in double reduction of the QB quinone, delivery of the second electron being accompanied by the uptake of two protons from the cytoplasm to form ubiquinol. The immediate products of light absorption, energy funnelling and charge separation are therefore one QH2, which dissociates into the intra-membrane phase, and two oxidised cyt c2 which can diffuse in the periplasmic space. These mobile products are then used as substrates by the bc1.Oxidation of QH2 takes place at a site near the periplasmic side of the membrane and is a bifurcated reaction, one electron being used to reduce cyt c2 by passage through the so-called high-potential chain formed by the Rieske Fe-S protein and cyt c1, accompanied by the release of a proton. A notable feature of this reaction is a large scale change in the conformation of the Rieske protein that moves the Fe-S centre away from the Qo site and towards the cyt c1 heme. This movement prevents the second electron from being passed to the Rieske Fe-S centre. Instead, accompanied by release of the second proton, the second electron is passed back across the membrane via two b-type hemes to a second quinone reductase site. Oxidation of a second QH2 at the Qo site results in the reduction of a second cyt c2 via the high potential chain, and double reduction and protonation of the quinone at the Qi site. As the sites for reduction/ protonation of quinone in both the RC and bc1 are located on the cytoplasmic side of the membrane, and the site for quinol oxidation in the latter is located on the periplasmic side, light-powered cyclic electron flow in this system is coupled to the translocation of protons from the cytoplasmic compartment into the periplasm.Summarises the movement of quinone and cyt c2 between binding sites on the RC and bc1, and the proton translocation that is powered by sunlight.

The RC acts in partnership with the bc1 to translocate protons across the photosynthetic membrane, transducingsunlight into the energyof the proton motive force. Dashed black arrows show the movement of reducing equivalents between the RC and bc1, or between the cytoplasmic and periplasmic side sides of the bc1, by the mobile carriers ubiquinol and cyt c2. Blue arrows show sites for the uptake of protons from the cytoplasm, and red arrows the

site of proton release into the periplasm. The migration of electrons internal to the RC and bc1 is not shown. The view of the bc1 dimer shows the Qi site of one monomer, and the Qo site of the second monomer.

Summaries of this process from the points of view of redox potential and free energy are. Transformation of the redox potential of P0 through light absorption triggers a cascade of redox reactions, as electrons flow via the QB site into the intra-membrane \sQ-pool, through the high potential chain of the bc1, and through the cyt c2 pool to re-reduce P0+. The additional energy of the initially-formed P0* excited state is progressively lost as charge is separated through the protein. However, part of this energy is preserved in the form of the energy of the pmf formed through the proton translocation that is coupled to electron flow. This pmf is then used to power a variety of energy- requiring reactions, includingATP synthesis, active transport, motion of the bacterial flagellum, and so on \sSphaeroides Photosystem.The formation of the excited state through light absorption transforms the redox potential of P0, triggering reduction of BAand subsequent electron transfer. 'cyt bc1' refers to the high-potential chain in the bc1 complex. Redox couples are written with the notation reactant/product, B shows free energy of charge separation in the RC.

Photosystem Diversity

The outline description given in the last section is based on the photosystem from Rba. sphaeroides, which has been the main workhorse for structural and functional studies. The photosystems from other species of purple bacteria operate on the same principles, but the details vary from organism to organism. So, for example, the first RC to yield a high resolution X-ray crystal structure, that from Blc. viridis, has an additional sub-unit comprising an extra-membrane tetra-heme cytochrome that is attached to the periplasmic side of the complex . One of the hemes of this cytochrome sub-unit acts as the initial reductant of the photo-oxidised primary electron donor in this RC, termed P0+, the water- soluble c-type cytochrome delivering electrons from the bc1 to this tetra-heme cytochrome sub-unit, and so only indirectly reducing P0+. This RC contains BChl b rather than BChl a, and has menaquinone in the QA quinone site.

Perhaps the greatest variety comes in the type and organisation of light- harvesting complexes present in purple bacteria. It has been known for many years that many species contain one or more types of peripheral light harvesting complex, the levels of which are affected by environmental factors such as light intensity or oxygen levels. In other species, including Blc. viridis and Rsp. rubrum, this peripheral antenna is absent, the light harvesting function being the sole responsibility of the LH1 antenna that surrounds each RC.A full understanding of the process of photosynthetic energy transduction in purple bacteria not only requires detailed information on the structure and mechanism of individual components, but also an appreciation of how these are put together to form a fully functioning photosynthetic membrane. In recent years the technique of atomic force microscopy has provided detailed information on this aspect of the photosystem, the distinctive ring-like topography of light harvesting complexes proving particularly amenable to imaging and interpretation. In Blc. viridis, which lacks peripheral antenna complexes, the RC-LH1 complexes form very regular arrays, each unit in the array comprising a central RC surrounded by a complete ring of LH1 pigment-protein.

In Rsp. photometricum, the RC-LH1 complex also has a closed ring of \s/ß pairs, but the presence of LH2 means that the membrane is less ordered, with individual RC-LH1 complexes separated by variable amounts of smaller rings attributable to the peripheral LH2 complex. In Rps. Palustris, the photosynthetic membrane is also disordered in this way, and

in agreement with the low resolution X-ray data described above, the ring of LH1 surrounding each Rps. Palustris RC is not complete, comprising only /ß pairs. In Rba. blasticus, two forms of the RC-LH1 complex are apparent, a minor monomeric form comprising an RC surrounded by an incomplete ring of LH1 pigment-protein, and a major dimeric form in which two RCs are surrounded by an S-shaped antenna, both with LH1 \s/ß pairs per RC. This dimeric arrangement was first reported for the Rba. sphaeroides RC-LH1 complex, and in this species RC-LH1 dimers are arranged in linear arrays several complexes in length, and surrounded by LH2 complexes. Although AFM has been successfully used to characterise the organisation of RCs and LH complexes in photosynthetic membranes from a variety of purple bacteria, the location of the bc1 and ATP synthase in these membranes has not been established. However, the information from AFM and kinetic spectroscopy has been used to build spectacular models of the photosynthetic membrane, which include speculations on the location of bc1 and ATP synthase.

DISCUSSION

The mechanistic or physiological significance of observed variations in the organisation of the photosynthetic membrane is not understood, but it may be linked, at least in part, to the metabolic flexibility displayed bythis group of bacteria. The species displaying the greatest flexibility, including Rba. sphaeroides and the closely related Rba. capsulatus, are capable of both photoautotrophy, and photoheterotrophy, and can also grow in the dark through aerobic respiration, or anaerobic respiration with a variety of terminal electron acceptors. Fermentative growth is also well documented, as is chemolithotrophy with hydrogen or thiosulphate as electron donors. Most purple photosynthetic bacteria can fix N2 to form NH3 and H2, and some can carry out denitrification of nitrate to N2. Some species are obligate anaerobes, but others can tolerate high levels of oxygen, although in species such as Rba. sphaeroides expression of the photosynthetic apparatus is down-regulated byoxygen. In another group, termed aerobic anoxygenic phototrophs, expression of the photosynthetic apparatus takes place onlyin the presence of oxygen. These bacteria are widespread with a number of intriguing characteristics, including low levels of RCs and LH complexes, repression of BChl synthesis by light, and high levels of carotenoid [7]–[9].

A major factor in the prominent use of Rba. sphaeroides as a model organism for studying purple bacterial photosynthesis is its metabolic flexibility. In addition to growing in the light, this species will grow in the dark in the presence of a suitable source of carbon and electrons, such as succinate or malate for example, and a suitable electron acceptor, such as oxygen. Although the expression of the photosynthetic apparatus is repressed at veryhigh levels of oxygen, a fully-functional photosystem is assembled at moderate-to-low oxygen tensions, although it is not required for growth. As a result, it is possible to introduce potentiallylethal mutations into the RC and LH complexes of the photosystem, and study the structural and functional consequences of these in proteins or membranes prepared from cells grown in the absence of light. Such experiments are not possible in obligate phototrophs, where impairment of the photosynthetic apparatus can lead to cell death, and incubation under illuminated conditions can give rise to reversion or suppression mutations.

Photosynthesis in Other Anoxygenic Photosynthetic Bacteria

Although by far the most heavily studied, the purple phototrophs are only one of several groups of bacteria that use BChl proteins to exploit sunlight as an energy source. The green non-sulphur bacteria, such as Chloroflexus aurantiacus, have a photosystem similar to that of purple photosynthetic bacteria. The RC of Cf. aurantiacus is similar to that of Rba. sphaeroides, with an intramembrane heterodimer of L and M polypeptides that scaffold the

electron transfer cofactors. However, this RC lacks the H-polypeptide, both quinones are menaquinone, and the bacteriochlorin cofactors comprise three BChls and three BPhes. The RC is associated with a B8-6 antenna complex that has spectroscopic similarities to the purple bacterial LH2 antenna, but shows sequence similarities to the polypeptides of the purple bacterial LH1. In addition to possessing an intra-membrane light harvesting system, green non-sulphur bacteria possess an extensive light harvesting system called a chlorosome, which is attached to the cytoplasmic face of the photosynthetic membrane. This comprises an aggregate of up to, 0 molecules of BChl c, with smaller amounts of protein, lipid and carotenoids, and the chlorosome transfers energy to the intra-membrane antenna via a set of baseplate proteins, and thence to the RC.

The RCs from purple bacteria and green filamentous bacteria are both classed as quinone-pheophytin type, or type-II RCs, along with the PS2 RC from oxygenic photosynthesis. Theremaininganoxygenic photosynthetic bacteria, the green sulphur bacteria and heliobacteria, contain a Fe-S type or Type-I RC similar to the PS1 RC from oxygenic photosynthesis. Again the reader is referred to the module by Yocum on RCs for a description of the PS1 RC; the key differences with the type-II RCs discussed thus far, is that the membrane-spanning electron transfer chain terminates in Fe-S centres rather than dissociable quinones, and within the RC pigment-protein the central electron transfer domain is flanked bytwo additional symmetrical domains that house antenna BChls or Chls and carotenoids. In green sulphur bacteria, such as Chlorobium limicola, the light harvesting function carried out by the intra-membrane antenna regions of the RC is again augmented by an extra-membrane chlorosome antenna comprising largely of BChls c, d, and e.

This is also connected to the intramembrane antenna via baseplate proteins, but the green sulphur bacteria also contain the Fenna-Matthews-Olson protein that participates in energy flow from the chlorosome to the intra-membrane components. The FMO protein is water-soluble, and was the first chlorophyll protein to be structurally characterised to a high resolution by X-raycrystallography.In the type-II RCs described above, the two quinones have discrete functional roles, the QA quinone acting as a one electron relay but the QB quinone being specialised for accumulation of two electrons and undergoing double protonation in order to generate a quinol. However, in the Type-I RCs the quinone cofactors are both non-dissociable, and on reduction pass the electron on to a Fe- S centre located on the symmetry axis. As a result, in principle, either branch of cofactors could beused to catalyse membrane-spanningelectrontransfer, and findings in recent years have shown that this is indeed the case [10]–[12].

CONCLUSION

The process of photosynthesis is most commonly associated with plants and algae, much of our understanding of the molecular basis for light energy capture and photochemical energy transduction has come from studies of photosynthetic bacteria. Photosynthesis, or the conversion of light energy into chemical energy in the form of ATP, is the utilisation of light as a source of energy for growth. Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the ‘halobacteria’. The final group of anoxygenic photosynthetic bacteria are the heliobacteria. These also have a Type-I RC, with a central electron transfer domain and two antenna domains, and unlike other photosynthetic bacteria this RC contains BChl g as a minor component. Heliobacteria donot have additional intra- or extra-membrane antenna complexes. Afeature of the heliobacterium RC, shared with the Type-I RC from green sulphur bacteria, is that it is formed from a homodimer of a single polypeptide, rather than the L/M heterodimer seen in purple bacteria. Another feature established in recent is years is that,

in common with PS1, both branches of membrane-spanning cofactors are used to transfer electrons across the membrane during photochemical charge separation.

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CHAPTER 4

BACTERIAL PHOTOSYNTHESIS: MECHANISM AND MICROBIAL STRESS RESPONSES

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ABSTRACT:

The existence of photosynthetic bacteria predates the time when the Earth's atmosphere could support human life. Yet, scientists have only just started to solve the puzzle of how these microscopic creatures carry out the processes of photosynthesis. Similar to how plants do it, oxygenic photosynthetic bacteria carry out photosynthesis. They emit oxygen, absorb carbon dioxide, and have pigments that capture light. The only category of oxygenic photosynthetic microorganisms now recognised is cyanobacteria or cyanophyta. Cyanobacteria do, however, come in a variety of species. They often have blue-green hues and are supposed to have helped transform the early oxygen-poor atmosphere of the Earth into an oxygen-rich environment, which is likely to have led to the variety of life on the planet. As a result of this change, the majority of anaerobic creatures that survived in the lack of oxygen ultimately went extinct and new species that need oxygen started to appear.

KEYWORDS:

Bacteria, Bacterial Photosynthesis,Microorganisms, Microbiology.

INTRODUCTION

Nowadays, photosynthetic bacteria are employed for a wide range of purposes, such as the bioremediation of pollutants, animal feed, biofertilizers, and water filtration. They are used in the purification of contaminated water since they can flourish and make use of hazardous compounds like H2S or H2S3 [1]–[3].Gram-negative, anaerobic, filamentous photosynthetic bacteria are sulphur bacteria. They are known as sulphur bacteria because they use hydrogen sulphide instead of water as an electron donor while converting carbon dioxide into carbohydrates.You will learn about photosynthetic bacteria that produce green and purple sulphur, photophosphorylation, and the non-cyclic and cyclic electron transport mechanisms in this unit.

Green Sulphur and Purple Photosynthetic Bacteria

Gram negative, anaerobic, filamentous photosynthetic bacteria are sulphur bacteria. They are known as sulphur bacteria because they use hydrogen sulphide instead of water as an electron donor while converting carbon dioxide into carbohydrates. S bacteria may be classified as green or purple bacteria depending on how much carotenoid they contain. Green germs show as yellow green, green orange, or brown, whereas purple bacteria appear as purple or reddish brown. As the human eye is unable to discern bacteriochlorophyll's absorption spectrum in the infrared area, its colour is not taken into consideration.

Both green and purple bacteria play a crucial biological role in the atmospheric sulphur cycle. To comprehend the function of these microorganisms, knowledge of sulphur cycle processes

is essential. Sulfur is found in nature in the free and coupled forms of the amino acids cysteine and methionine as well as in its organic and inorganic states. S bacteria are responsible for converting sulphur between its oxidised and reduced states. They convert H2S to sulphate, which is the best kind of necessary sulphur that plants can use. Certain S bacteria also engage in anaerobic sulphur oxidation in conjunction with nitrate reduction. Plants and soil-dwelling microorganisms decrease and absorb sulphate. In aerobic circumstances, assimilated sulphate is incorporated into proteins. With the creation of H2S, dissimilatory sulphate reduction takes place. Sulfate serves as the terminal electron acceptor in the reduction of sulphate to hydrogen gas through sulphite. In lit, anaerobic aquatic habitats, purple and green S bacteria coexist.

Biological Response to Stress

The bacterial stress response helps microorganisms to endure challenging and erratic environmental circumstances. Several bacterial processes identify various environmental changes and build the proper defences. A bacterial cell may respond to several stressors at once, and the diverse stress response mechanisms communicate with one another via a maze of international regulatory networks.

Bacteria can endure a variety of environmental conditions, but in order to adapt to these challenging and changing circumstances, they need to be able to detect the changes and mount the proper gene expression and protein activity responses. A sophisticated network of components works together in bacteria's stress response to counter the external shock. Bacteria may respond to many stressors at once, and different stress response mechanisms interact with one another. A coordinated and efficient reaction is produced through a complex web of international regulatory systems. These regulatory mechanisms control the production of additional effectors that reliably preserve cellular homeostasis under a variety of circumstances.The virulence of pathogenic organisms may be significantly influenced by stress response mechanisms.One of the most important stress response mechanisms in bacteria is the heat shock response, which is regulated by the sigma factor sigma.

1. The Cpx two-component system and the sigma factor sigma E primarily regulate the envelope stress response.
2. The cold shock response, which controls RNA chaperone and ribosomal factor expression

Osmotic Stress An abrupt shift in the solute concentration surrounding a cell results in a rapid alteration in the transport of water across the cell membrane, which is known as osmotic shock or osmotic stress. Osmosis draws water out of the cells when there are significant quantities of salts, substrates, or any other solute in the supernatant. Moreover, this prevents the entry of cofactors and substrates, stunning the cell. In contrast, water enters the cell in significant volumes at low solute concentrations, causing the cell to enlarge and either rupture or go through apoptosis. the abrupt drop in osmotic pressure that causes bacterial or other cells to burst in a solution. In order to liberate cellular components for biochemical investigation, osmotic shock is sometimes generated.

Osmoregulation

The process of maintaining the equilibrium of salt and water across membranes inside the body is known as osmoregulation. Water, electrolytes, and nonelectrolytes make up the fluids within and around cells. A substance that separates into ions when dissolved in water is known as an electrolyte. In contrast, anonelectrolyte does not split into ions in water. Blood plasma, fluid found within cells, and interstitial fluid, which is present in the gaps between the body's cells and tissues, are all examples of bodily fluids. The body's membranes may

pass through each other partially. Cell membranes are normally impermeable to solutes, however semipermeable membranes are permeable to certain kinds of solutes and to water [4]–[6].

The human body is not an island. Water and electrolytes are continuously introduced into the system. To maintain the osmotic equilibrium, extra water, electrolytes, and wastes are carried to the kidneys and eliminated. The kidneys save fluid as a consequence of insufficient fluid intake. By consuming food and water and excreting waste in the form of perspiration, urine, and faeces, biological systems continually interact and exchange water and nutrients with the environment. There is a propensity for toxic waste and water to collect, which may have dangerous repercussions when there is no mechanism to control osmotic pressure or when a disease impairs this system.In addition to controlling the general osmotic pressure across membranes, mammalian mechanisms have developed to control particular concentrations of significant electrolytes in the body's three main fluid compartments: blood plasma, interstitial fluid, and intracellular fluid. As water moves across membranes to control osmotic pressure, the volume of the fluid compartments may also momentarily alter. Osmotic pressures have a direct impact on blood pressure since blood plasma is a fluid.

The existence of photosynthetic bacteria predates the time when the Earth's atmosphere could support human life. Yet, scientists have only just started to solve the puzzle of how these microscopic creatures carry out the processes of photosynthesis.Similar to how plants do it, oxygenic photosynthetic bacteria carry out photosynthesis. They emit oxygen, absorb carbon dioxide, and have pigments that capture light. The only category of oxygenic photosynthetic microorganisms now recognised is cyanobacteria or cyanophyta. Cyanobacteria do, however, come in a variety of species. They often have blue-green hues and are supposed to have helped transform the early oxygen-poor atmosphere of the Earth into an oxygen-rich environment, which is likely to have led to the variety of life on the planet.

As a result of this change, the majority of anaerobic creatures that survived in the lack of oxygen ultimately went extinct and new species that need oxygen started to appear. Nowadays, photosynthetic bacteria are employed for a wide range of purposes, such as the bioremediation of pollutants, animal feed, biofertilizers, and water filtration. They are used in the purification of contaminated water since they can flourish and make use of hazardous compounds like H2S or H2S3.Gram-negative, anaerobic, filamentous photosynthetic bacteria are sulphur bacteria. They are known as sulphur bacteria because they use hydrogen sulphide instead of water as an electron donor while converting carbon dioxide into carbohydrates.You will learn about photosynthetic bacteria that produce green and purple sulphur, photophosphorylation, and the non-cyclic and cyclic electron transport mechanisms in this unit.

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Contrary to popular belief, green bacteria are neither blue-green algae or cyanobacteria, but rather a separate kind of phototrophic bacteria belonging to the Chlorobiaceae family.Bacteriochlorophylls c, d, or e, as well as the carotenoids chlorobactene, hydrochlorobactene, isorenieratene, and â-isorenieratene, are examples of photosynthetic pigments. As electron donors, they utilise H2S, other reduced inorganic sulphur compounds, and H2. One intriguing fact is that, while growing with H2 as an electron donor and also vitamin B, they cannot utilise sulphate as a source of sulphur and instead need sulphide to satisfy biosynthetic demands. Prior to being converted to sulphate, the elemental sulphur generated by H2S oxidation is deposited extracellularly as sulphur globules. In the absence of an inorganic reductant, they are unable to develop photoheterotrophically utilising organic substances as their primary or only carbon source. Acetate or pyruvate may be photoassimilated by them, but only when H2S and CO2 are present simultaneously.

The Calvin-Benson cycle is used by other bacteria; they are the only bacteria that employ the reductive tricarboxylic acid cycle. Chlorobium, Chloropseudomonas, Pelodictyon, Clathrochloris, and Chlorobacterium are significant genera of green S bacteria. Anaerobic environments also include green Chloroflexaceae bacteria that don't produce sulphur. In terms of structure, nutrition, metabolism, and ecology, they are distinct from green S bacteria, however they both possess bacteriochlorophyll c or d as primary or minor pigments, respectively. They often live in hot springs with little to no organic matter and are photoheterotrophs, facultative photoautotrophs, or chemoheterotrophs. They constantly grow alongside cyanobacteria and get their organic resources from those organisms. Chloroflexus, Oscillochloris, Chloronema, and Heliothrix are some of the principal genera. Only the hot springs of Western Oregon and Yellowstone National Park contain Heliothrix.

The Chromatiaceae family of strictly anaerobic, photoautotrophic purple S bacteria inhabit sulfide-rich waters. Sulfate-reducing bacteria including Desulfovibrio, Desulfococcus, Desulfomonas, Desulfosarcina, Desulfolobus, Desulfobacter, and Desulfococcus spp. produce sulphur dioxide as a byproduct. Bacteriochlorophyll an or b, carotenoids of groups 1, 3, and 4, are photosynthetic pigments. Molecular hydrogen, thiosulfate, sulphur, H2S, and organic molecules all function as electron donors. In all purple S bacteria, photosynthetic nitrogen fixation occurs. They may heterotrophically grow on pyruvate in the dark. The Calvin-Benson cycle converts H2S to sulphate by oxidising it with elemental sulphur. It invariably results in a brief buildup of elemental sulphur as sulphur globules, which are cytoplasmic gas vacuoles. Granules of poly-â-hydroxybutyrate are also produced by them. Some bacteria also exhibit unique infoldings or incursions in their cell membranes. In order to accommodate additional centres of respiration and photosynthetic activity, the membrane area enlarges as a consequence.

Thiospirillum, Chromatium, Ectothiorhodospira, Thiocystis, Thiocapsa, Lamprocystis, Thiodictyon, Thiopedia, and Amoebobacter spp. are the principal genera. Moreover, there are purple nonS bacteria that are H2S sensitive. These are principal members of the Rhodospirillaceae family genera Rhodospirillum, Rhodopseudomonas, Rhodomicrobium,

Rhodopila, Rhodocyclus, and Rhodobacter. In the presence of light, they anaerobically oxidise sulphide at extremely low concentrations. They generally exist in freshwater settings where organic matter is plentiful and sulphide is absent or present in very small amounts. A broad variety of organic molecules, including fatty acids, primary or secondary alcohols, organic acids, carbohydrates, proteins, and aromatic compounds, may be photoassimilated by them. In order to create reduced and mixed forms of nitrogen to sustain cell development, certain species simultaneously engage in simultaneous denitrification and nitrogen fixation activities.

The Value of Sulfur Oxidation and Reduction for the Environment

Despite the fact that sulphur is plentiful in nature, plants can only use its oxidation products like sulphate. For utilisation by plants, sulphate anions solubilize inorganic salts containing nutrients like phosphorus. Furthermore, sulphate reduces ammonia synthesis by microbes that results in excessive alkalinity. Sulfur anaerobic oxidation and nitrate reduction are sometimes harmful processes that reduce soil fertility. Very hazardous to aquatic flora and wildlife, H2S generated by sulphur reduction is often linked to fish death. H2S may harm plants in anaerobic soils with standing water, such paddy fields. In order to sustain the development of H2S aerobic chemoautotrophs or anaerobic photoauto- and photoheterotrophs, H2S generated during reduction is a significant source of reducing power. Sulfureta environments like sulfate-rich stagnant lakes are dominated by sulfur-reducing bacteria and phototrophic bacteria. These bacteria encourage the synthesis of elemental sulphur from sulphates; their actions result in the creation of geological sulphur deposits. S bacteria also help to cause metal pipes to corrode.The red molecules in the accompanying illustration are moving electron shuttles. The emphasis is on protein complexes with several electron transfer stages.

Photophosphorylation

Photophosphorylation refers to the phosphorylation of ADP to produce ATP during the photosynthesis process. Living things can only get their energy from sunlight and reduction-oxidation processes. The universal energy currency of life, ATP, is produced by all organisms. Typically, photolysis, or photodissociation, of water and a constant one-way flow of electrons from water to PS are involved in photosynthesis.

Light energy is employed in photophosphorylation to produce a high-energy electron donor and a lower-energy electron acceptor. Then, electrons travel haphazardly through an electron transport chain from source to acceptor.ADP is converted to ATP by a process known as photophosphorylation, which replenishes the universal energy unit that all living things utilise. In the most basic prokaryotic systems, photosynthesis serves just to generate energy and not to assemble any living molecules. But, in plants, the process of photosynthesis also generates energy in the form of reduced coenzymes that power the biochemical synthesis process, which results in the creation of glucose and other sugars. Both Photosystem II and Photosystem I work in sequence throughout this procedure. From Moore, depicts some of the phases involved in non-cyclic electron transport.

Consider the electron energies measured in units of the redox potential in volts as another method to frame this two-stage process for supplying the energy for constructing molecules. This diagram, which is based on Karp's work, shows the phases in the process with the degree of energy obtained being shown by the position on the vertical axis.The electron transport interactions that provide the light energy for chemical reactions take place at the thylakoid membranes.

Photosynthesis's Cyclic Electron Transfer

ADP is converted to ATP by a process known as photophosphorylation, which replenishes the universal energy unit that all living things utilise. In the most basic prokaryotic systems, photosynthesis serves just to generate energy and not to assemble any living molecules. ADP is converted to ATP for the cells' immediate energy in these systems via a process known as cyclic photophosphorylation. Just Photosystem I and chlorophyll P0 are used in this process.

Aerobic To Anerobic Transitions

All organisms must adapt their physiology in response to variations in oxygen supply in order to survive. Nevertheless, a systems level understanding of the sequence of events and the regulatory mechanisms that control how and when changes in ambient oxygen tension result in an adequate cellular response is lacking. In order to create a temporal model that depicts the cellular processes that drive the transition between the organism's two opposing cell states of anoxic quiescence and aerobic growth, changes in the transcriptome, proteome, ATP, and growth were examined in a halophilic archaeon. This model predicts that upon oxygen inflow, a fast burst of protein synthesis precedes the stimulation of ATP and transcription. This quick exit from anoxic quiescence is followed by the restart of growth. This concept also implies that cells in a quiescent state seem to be actively prepared to produce energy from a number of sources. Keygene connections between transcription and translation in a dynamic temporal analysis point to a number of critical processes for cellular survival in anoxia as well as particular examples of post-transcriptional control.

It is necessary to collect extensive and precise measurements of changes in parameters, such as transcription, translation, and metabolism, in order to comprehend cellular responses to oxygen at the molecular systems level. The identification of the whole microbial proteome and metabolome is currently limited by technology; for instance, the greatest reported coverage for microbial shotgun proteomics is %. In contrast, transcriptome measurements are fairly thorough. The dynamic nature of information processing at each of these levels further complicates the collective comparative study of overall changes in the transcriptome, proteome, and metabolome, in addition to this discrepancy in technological tractability.

Microbes that are facultative, like E. coli and S. enterica, have the ability to change their metabolism to support growth in either aerobic or anaerobic environments. Alterations in the pace, path, and effectiveness of electron transport channels occur along with the switch from aerobic to anaerobic metabolism. The fundamental E. coli routes for aerobic vs anaerobic electron transport. Pyruvate is transformed into formate, acetate, or ethanol, as well as CO2 and H2 gas when it is subjected to anaerobic conditions without other electron acceptors. Yet, when other electron acceptors are accessible, the options and energy yield increase. Even in an aerobic environment, E. coli may create the two different cytochrome oxidases, cytochrome o and cytochrome d, which are formed in high and low oxygen environments, respectively.

DISCUSSION

It is described how oxidative stress, which is characterised as an imbalance between the formation of reactive oxygen species and antioxidant defences, may contribute to tissue damage in diabetes mellitus. Major antioxidant defence systems are covered together with descriptions of significant free radicals and their biological origins. Examples of potential free radical damage effects are given, with a focus on lipid peroxidation. Lastly, the issue of whether diabetes mellitus causes an increase in oxidative stress is addressed [7]–[9].

Radical Freethinkers

A molecule containing oxygen that has one or more unpaired electrons and is thus extremely reactive with other molecules is referred to as a free radical.While most oxygen by-products are not very reactive, some of them may be converted into these highly reactive oxidants via biological system metabolism. Reactive oxygen species are not all detrimental to the body. Several of them are effective in eliminating diseases or germs that have invaded.Yet, in order to become stabilised, free radicals may chemically interact with elements of the cell such as DNA, protein, or lipid and steal their electrons. This subsequently destabilises the molecules that make up the cell, causing them to seek out and grab an electron from another molecule, which starts a long chain of free radical reactions.

Antioxidants

Any cell that uses oxygen and enzymes to carry out its operations is subject to oxygen free radical reactions that might seriously harm the cell.Antioxidants are substances that exist in cells that stop these processes by giving the free radicals an electron without becoming unstable themselves. The root cause of oxidative stress is an imbalance between oxidants and antioxidants.

Damaged Resulting from Oxidative Stress

Many pathophysiological disorders in the body are caused by oxidative stress. Neurodegenerative conditions including Parkinson's disease and Alzheimer's disease, malignancies caused by gene mutations, fragile X syndrome, atherosclerosis, heart failure, and heart attacks, as well as inflammatory illnesses are a few of these.

Cross-Protection

Cross-protection is the capacity of one stress state to provide defence against another stress. Several studies have shown that adaptations to acids provide resistance to a variety of stress factors, including as heat, salt, H2O2, crystal violet, and polymyxin B. Yet, adapting to other pressures seldom results in appreciable acid tolerance. This suggests that heat, salt, and H2O2 may be more particular stress signals, but exposure to acid may be regarded by bacteria as a more broad stress indication. The fact that acid shock generates cross-resistance to a number of stressors has also led to evidence suggesting cells going through acid shock in the stomach will be well equipped to withstand the environmental pressures they would later face in the intestine.

The pathogenicity of low pH

Many studies have emphasised the significance of low pH in the pathogenesis of enteric bacteria. For instance, buffering stomach acidity greatly reduces the infectious dosage for Salmonella species, indicating that the. The greater the organism's ability to withstand stomach acid, the greater the likelihood that it will live and spread illness. Moreover, the low pH that develops in phagocytic compartments after the invasion of epithelial cells and macrophages triggers the systems involved in Salmonella pathogenesis. S. typhimurium is also attenuated by a number of mutations that increase acid sensitivity. Poynter et al. have shown that surface attachment of the bacterium increases acid tolerance in E. coli. In reality, the ability of enterohemorrhagic E. coli to survive in acid has been linked to the disease's apparent low infectious dose. It is undeniably true that the pathogenic process and enterobacteria's reaction to acid stress are related.

Response to Thermal Stress and Heat Shock

Many experiments have been done where stress was applied to the cells in the form of heat shock, or by subjecting them to high temperatures.Prokaryotes only have one copy of a heat shock gene, which is a known fact.The majority of prokaryotes' regulatory stress genes must be expressed constitutively under all circumstances due to this fact and the critical role that most heat shock genes play. Yet, eukaryotes typically have at least two copies of the heat shock gene. One of the two is controlled by heat shock regulation, while the other is controlled by constitutive factors.

Mechanism for Stress Gene Induction, Regulation, and Control

The rpoH gene controls the heat shock regulon in prokaryotic cells. A KD sigma factor is the end result. For instance, it has been discovered that the rpoH gene, which codes for heat shock factor, regulates the main heat shock genes of E. coli. The main RNA polymerase enzyme is directed by the factor to identify the promoters for heat shock genes.

Starvation Stress and Nutrient Stress

For the majority of bacteria, nutrient deprivation and other environmental challenges are common occurrences. True "feast" or non-stress circumstances are hard to come across for microorganisms outside of the lab. As a result, bacteria are often found under conditions of nutritional deprivation, stress-induced sluggish development, or no growth at all. Luckily, this is the case because, given the rates of bacterial growth attained in the lab under no-limiting settings, the effects would be fatal if microbial growth were not limited in natural or animal host habitats.

Hunger-Stress Reaction

It should be highlighted that starving cells and so-called stationary-phase cells need different treatment. In contrast to starved cells, which occupy cultures that have stopped growing in response to depletion of one or more specific nutrients, stationary-phase cells often inhabit cultures that have stopped growing after exponential growth in rich or non-limiting conditions. Whereas the constraint or stress that inhibits development in stationary-phase cells is often not well-defined and not restricted to a single stress, it is well-defined in starved cells [10]–[12].

CONCLUSION

The fact that stationary-phase cultures often acquire a significantly larger cell density compared to starving cultures is another important distinction. This may have a considerable impact on cellular responses generally and long-term survival. Moreover, there may or may not be gene or protein overlap between cells in stationary phase and cells that have been deprived. The SSR especially relates to how starving cells react. When deprived of a necessary resource, such as a carbon-energy source, non-differentiating microorganisms like E. coli, Salmonella, and others react by stimulating the production of up to new or preexisting proteins. The starvation-stress response involves a genetic and physiological remodelling that takes place. The SSR serves to enable the long-term starvation survival of the bacteria and to offer a general cross-resistance to a variety of other environmental stresses, such as exposure to reactive oxygen and nitrogen species, antimicrobial peptides/proteins, extremes in temperature, pH, and osmolarity, as well as exposure to these species.

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CHAPTER 5

EXPLORING THE IMPORTNACE OF NITROGEN METABOLISM

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ABSTRACT:

The main characteristics of an organism, such as function and structure, are defined by the polymeric nitrogen-containing molecules, such as proteins and nucleic acids, via nitrogen metabolism. Proteins contribute the structure, functionality, and mechanism of metabolic processes. In nucleic acid polymers, genetic information is stored. There is a distinct metabolic route for each monomer of these macromolecules. Moreover, monomeric nucleotides serve as crucial intermediates in all metabolic pathways and second messenger molecules, often in the form of cyclic nucleotides, which are both crucial for energy turnover. Ammonia NH3, in its neutral or charged state, NH +, is recycled in all nitrogen metabolic processes. Ammonia, however, is not a significant type of nitrogen on Earth and must be supplied to meet the rising need of living forms. The necessity for tiny organic and inorganic molecules as raw material for a bacterial culture to develop, however straightforward it may appear, cannot be overstated. Atmospheric N2 is ultimately the source of NH3.

KEYWORDS:

Nitrogen Metabolism, Bacterial Growth, Microbiology, Acid Polymers.

INTRODUCTION

Some bacterial species, including the symbiotic eubacteria Rhizobium and the archaea cyanobacteria, include an enzyme complex for the conversion of molecular nitrogen to ammonia, a process known as nitrogen fixation. This complex, called nitrogenase, is necessary for the transfer of electrons from ferredoxin to N2 and comprises the cofactors Fe-S and Mo-Fe [1]–[3].

The Process of Nitrous Oxide Metabolism

One of the most common macro-elements that affects plant development, particularly in agricultural settings, is nitrogen, along with carbon, hydrogen, and oxygen. For the creation of nitrogenous organic molecules, the atmosphere is the primary supply of nitrogen.Nucleic acids, proteins, certain phytohormones, and several vitamins are examples of important biomolecules where it may be found. In the majority of biochemical processes that contribute to life activities, nitrogen, a component of these biomolecules as well as many other chemicals, is involved.Nitrogen is essential for plant metabolism and structure; hence plants need larger concentrations of it. Nitrogen makes up around % of the earth's atmosphere, soaking the whole plant kingdom, yet most plants are unable to use it in its purest form. They must rely on the soil for its supply, and from it, they get nitrogen in inorganic form as nitrate or ammonium molecules[4]–[6].

The direct utilisation of atmospheric nitrogen is limited to a few types of prokaryotic systems.

One or two prokaryotic life forms in a stable ecological system transform atmospheric nitrogen into a form that can be supplied to higher plants and animals.The most beneficial form of ammonia, glutamine, and other nitrogen-containing chemicals needed for plant development and maintenance, are first converted from ammonia, which is then transformed into glutamate and glutamine.

Oxygen Cycle

In addition to agricultural manure and nitrate, additional sources of nitrogen in soil include ammonium ions, organic nitrogen, and ammonium nitrate. In order to be used for metabolism, nitrate is changed into ammonia. The roots of plants may directly absorb ammonium ions and organic nitrogen in the form of amino acids that have been mobilised from proteins or proteins that have partly been degraded.When organic systems die and decompose, amino acids, purines, and pyrimidines are cycled back to ammonia. However, part of this ammonia is removed from the nitrogen cycle during the process of converting it to NO2, nitrogen gas, or NO3. As a result, both catabolic and anabolic mechanisms contribute to nitrogen metabolism.

The three anabolic processes are: protein synthesis, nitrogen fixation, and amino acid synthesis.Proteolysis and amino acid oxidation, denitrogenation, and nitrification are the catabolic processes. It is clear from this that nitrogen is present in the environment in a variety of forms for the plant system, including nitrate, ammonia, organic nitrogen, and molecular nitrogen. The nitrogen cycle is made up of physical and biological processes that continuously interconvert various forms to maintain a constant level of atmospheric nitrogen.

1. Nitrification and ammonium nitrate
2. Denitrogenization;
3. Nitrogen fixation

Both Nitrification and Ammonification

Ammonification refers to soil bacteria converting organic nitrogen to NH+. Animal excrement, together with decomposing plant and animal remnants, are the sources of the organic nitrogen in soil. During a few days of its creation, ammonia undergoes further oxidation, first producing nitrite and subsequently nitrate in warm, wet soils with an approximate pH of neutral. The process of this oxidation is called nitrification. These two procedures are carried out by certain bacteria.The Bacillus species, B. mycoides, B. vulgaris, and B. ramosus, are saprophytic bacteria that ammonify. In addition to releasing ammonia from organic substances found in nature, certain fungi and actinomycetes can do the same. Nitrification is accomplished by chemoautotrophic bacteria belonging to the Nitrosomonas and Nitrobacter genera.Only nitrite is produced by Nitrosomonas' conversion of ammonia, and Nitrobacter subsequently uses this nitrite to produce nitrate. Both processes are exergonic, and the bacteria use the energy created during the oxidation of either nitrite or ammonia to power their chemosynthesis.

Taking Up Nitrates

Higher plants' root systems absorb nitrate, the most prevalent type of nitrogen in the soil, as NO -. In order to be incorporated into the nitrogenous compounds, it must first be reduced to the NH3 level since it cannot be utilised by plants directly.The nitrate reductase enzyme transforms nitrate for the first time into nitrite, a process that requires respiratory energy.From bacteria including E. coli, Pseudomonas aeruginosa, Neurospora, and soybean leaves, among other sources, the nitrate reductases have been purified. This metalloenzyme is

a complicated homodimerizer. Both nitrate and NAD H have binding sites for it.In order to support the following sequence of electron transfer processes, three cofactorsFAD, heme-Fe, and molybdenum cofactorform redox centres: The molybdenum ion MoCo is a compound of the organic molecule pterin, a metal chelator. There are amino acids in each nitrogen reductase subunit, which includes all three cofactors.While some plants utilise NADPH or NADH, most plants use NADH. The nitrate reductase genes have been cloned from a number of higher plants. In order to further reduce nitrite to generate NH3, the procedure is repeated using the intermediates hyponitrite and hydroxylamine.Two more electrons are added by decreased NAD+ at each stage. The two functional domains are connected by a sulphur ligand during this conversion of NO to NH and insertion into the molecule. Coordination with NR is used to control NiR's transcription. Cells need enough NiR to completely eliminate all of the nitrite that is created by NR since nitrite is harmful.

De-Nitrification

De-nitrification, also known as nitrification, is the process of turning nitrate and nitrite into ammonia, nitrogen gas, and nitrous oxide. The nitrogen cycle is finished when the atmosphere is filled with gaseous nitrogen. Anaerobic bacteria like Pseudomonas denitrificans and Bacillus subtilis decrease nitrates in this process by a sequence of reactions to ammonia and free nitrogen. The bacterium Thiobacillus denitrificans. Azotobacter, Clostridium, and Micrococcus, among others.

Fixing of Nitrogen

The process of turning basic di-nitrogen into an organic form and making it accessible to plants is known as nitrogen fixation. Based on the finding that this procedure generated a superior yield if the non-leguminous crops grew after the leguminous crops, the rotation of leguminous and non-leguminous field crops became a staple of traditional agricultural practise.However, no one understood that the advantage was connected to the fixation of atmospheric nitrogen until Baussingault demonstrated in that soil fertility increased because of some bacteria found in soil and in the root nodules of leguminous crops, and that these bacteria were capable of fixing atmospheric nitrogen.There are two different ways that nitrogen may be fixed: biologically and non-biologically.

Nitrogen Fixation Using Non-Biological or Physical Means

A molecule made of nitrogen is very stable. With a triple bond between two nitrogen atoms, it is present as di-nitrogen. In terms of length, ionisation potential, and stretching frequency, the nitrogen bond is the shortest, measuring 1.5. It has a high level of resistance to chemical assault because of this.It takes roughly 5 kcal of energy to break this triple bond, but it's incredibly challenging to do so. Nitrogen is converted to ammonia in the fertiliser sector by being heated to very high pressures and temperatures while passing through an iron catalyst.During electrical discharges caused by lightning, nitrogen may also be fixed. The nitrogen in the atmosphere reacts with the oxygen in the air to create nitrogen oxides, which are then hydrated by water vapour and transported to the ground as nitrites and nitrates by rain.

Biological Nitrogen Fixation

Certain bacterial species have evolved the ability to convert nitrogen to ammonia throughout the course of evolution; this conversion is controlled by a group of genes known as the nitrogen fixing genes. As "nitrogen-fixing" organisms, these species are known. These creatures may be either bacteria that live in symbiosis with higher plants or non-symbiotic

microbes that can exist on their own.Both aerobic and anaerobic heterotrophic bacteria, photosynthetic bacteria, and some blue-green algae are included in the former category.The Rhizobium bacteria constitute a significant nitrogen-fixing cooperative in the symbiotic system, which also includes several members of the Leguminosae family, including peas, beans, clovers, soybean, and others.The growth of nodules on plant roots is a crucial aspect of the symbiotic fixation.

Enzyme Nitrogenase

Living things include many different kinds of complexes, enzymes, and other substances that might be vital to their existence.Some bacterial species manufacture an enzyme called nitrogenase that is essential to their survival and expansion. The unique structure and symmetry of nitrogenase, an enzyme with a vital role exclusive to the bacteria that use it, as well as its sensitivity to other substances that might stop it from working, make it an enzyme that is only found in these particular bacteria. A set of enzymes that allows atmospheric nitrogen to be fixed is what it is. As much study has been conducted on this enzyme, the specific structure of nitrogenase is nearly entirely understood. In addition to binding to nitrogen gas, nitrogenase may also attach to other substances, which can hinder and lessen how much ammonia is produced for the remainder of the organism. Without appropriate operation, the bacteria that use nitrogenase would not be able to live, and other creatures that rely on these bacteria would also perish.

In only being able to fix nitrogen, nitrogenase is able to make nitrogen more reactive and useable in other processes that support the growth and well-being of organisms. However because of the stability of the molecule and its inert nature, living things find it challenging to utilise nitrogen gas, or N2, as a source of nitrogen. N2 is a gas that is stabilised by a triple bond. Nitrogen gas, N2, is separated using nitrogenase, which then converts it into ammonia, NH3, throughout the procedure.Organisms have a more reactive supply of usable nitrogen in the form of ammonia, which they may utilise to produce proteins and nucleic acids, both of which are essential for the organism. Molybdenum nitrogenase, vanadium nitrogenase, and iron-only nitrogenase are the three varieties of nitrogenases that are known, according to Peters and Szilagyi, and the molybdenum nitrogenase's crystal structure is the one that has been investigated the most of the three. In order to produce two ammonia molecules and hydrogen gas for each molecule of nitrogen gas, the nitrogenase enzyme splits a diatomic nitrogen gas molecule utilising a lot of ATP and 8 electrons. Because of this, the bacteria that use this enzyme must use a large portion of their energy as ATP in order to continuously get a consistent supply of nitrogen. Organisms couldn't survive without nitrogenase because they wouldn't have a supply of nitrogen for other critical activities. Nitrogenase fixes nitrogen gas into ammonia, which is essential for other reactions.

The workings of nitrogenase have been studied in great depth over the years, however the structure of nitrogenase has not yet been fully determined. The homocitrate molecule, a histadine amino acid, a cysteine amino acid, an iron-molybdenum cofactor centre, sulphur, and molybdenum are all components of the nitrogenase enzyme.As a result of its sensitivity to other substances, nitrogenase cannot be used readily by other species. If oxygen gas were to oxidise nitrogenase, it may cause numerous iron atoms to break, which might cause damage. Acetylene and carbon monoxide may also be joined by nitrogenase, making all three molecules strong competitors for the enzyme's substrate binding site. Only anaerobic species can employ nitrogenase because of its limited functioning. A member of the nitrogenase-using anaerobic family of bacteria is called rhizobium, which is typically found in plant roots. In order to create nitrogen, they coexist with leguminous plants in a symbiotic connection in return for other nutrients and defence against oxygen and other substances that can impair

nitrogenase's function. While nitrogenase's drawbacks prevent it from being widely used by organisms, they are a crucial component of the bacteria that do.

The bacteria that use nitrogenase depend on this particular enzyme to function. Since it serves as the majority of the nitrogen used by anaerobic bacteria, its function is essential. More organisms don't produce their own nitrogen source, though, due to the high cost of ATP and electrons, which is another constraining factor. Nitrogenase is an asymmetric protein due to the specific connections that make up its structure. Before we fully comprehend nitrogenase and its structure, more study is required, for example to determine whether or not nitrogenase contains a central atom and if so, what that atom is. The sensitive enzyme nitrogenase, which is responsible for fixing nitrogen, is quickly inhibited by oxygen and other substances. Because these bacteria can only obtain nitrogen from nitrogenase, nitrogenase is only found in anaerobic bacteria. The only enzyme that is absolutely necessary to the organisms that use it is nitrogenase, which is just one among countless other enzymes and complexes.

Symbiotic and Free-Living Bacteria's Physiology of Nitrogen Fixation

Symbiotic N2-Repair Bacteria

Symbiotic bacteria are those that form associations with either plants, animals, or fungi, and in these relationships, both the bacteria and the host are benefited. For instance, the bacteria Rhizobium form associations with the root nodules of higher plants, and the plants both provide the bacteria with shelter and aid in nitrogen fixation.It is advantageous for both the heterotrophic bacteria and their host plants that they fix di-nitrogen gas from the atmosphere in plant root nodules. Both companion plants and the crop that was planted after them in the same soil benefited from legumes. The formation of root nodules is clearly the cause of the legume's ability to fix atmospheric nitrogen.

Symbiotic bacteria initially start by infecting root hairs, resulting in an invasion inward through several cells. Auxin, a phytohormone made by the contaminating bacteria, may be the reason why neighbouring plant cells multiply quickly.A pigment that carries oxygen, meta-hemoglobin, is created as the bacteria enter the nodule cells and form enclosing membranes. It's possible that the substance that resembles haemoglobin acts as an oxygen sink or trap to keep the bacteria in an anaerobic state, which is essential for N2 fixation.Nitrogenase enzymes are responsible for fixing di-nitrogen. Activation energy is decreased by this enzyme. In stages of reduction, the fixation converts dinitrogen into 2NH3 by passing through the ambiguous intermediates HN=NH and H2N-NH2.

DISCUSSION

Last but not least, the ammonium is converted into some organic substances, including amino acids. As soon as the nitrogen is bound to the enzyme, all of this will happen. A bacterium's lifespan can be as brief as a few hours, and a portion of the bacterial population is constantly decomposing, dying, and releasing NH + and NO - ions for the host plant to use. The bacteria excrete the majority of the nitrogen that is fixed, and this nitrogen is then made available to the host plant and other plants in the area through the host plant. The Rhizobium genus includes the well-known symbiotic bacteria [7]–[9].To guarantee the presence of nitrogen-fixing organisms, symbiotic heterotrophic bacteria frequently present in the crop seed are frequently applied, or inoculated, in a dried powder form. Not every legume will become infected by the same type of bacteria. It has been discovered that Sesbania rostrata forms nodules in both its roots and stems, making it the most crucial host plant for the symbiotic N2-fixation process.

N2-Fixing Non-Symbiotic Bacteria:

It is not necessary for a host plant to support the non-symbiotic nitrogen-fixing bacteria. When exposed to the atmosphere, soil's nitrogen content was found to increase, according to Winogradsky's observations in.An increase in the nitrogen content of soil was discovered to be caused by the anaerobic bacterium Clostridium pasteurianum. A free-living aerobic bacteria called Azotobacter chroococcum was shown to be able to fix atmospheric nitrogen in by Beijerinck.Granulobacter, a different bacterial species, gets its nitrogen from the air directly. Because soil types vary, so do the amounts of atmospheric nitrogen that these bacteria are able to fix.The N2-fixer Azotobacter beijerinckia, which can withstand acid, is most prevalent in aerobic soils of tropical climates. The non-symbiotic fixation of N2 by Azospirillum species aids in the growth and yield of numerous crops.

CONCLUSION

The main characteristics of an organism, such as function and structure, are defined by the polymeric nitrogen-containing molecules, such as proteins and nucleic acids, via nitrogen metabolism. Proteins provide the structure, function, and mechanism of metabolic pathways. The monomeric nucleotides play crucial roles in all metabolic pathways as key intermediates and as second messenger molecules, frequently in the form of cyclic nucleotides, which are both crucial for energy turnover. Certain plants, such as Digitaria, water fern, azolla, and Gunnera macrophylla, have recently been discovered to have symbiotic relationships with various N2-fixing bacteria, including blue green bacteria. The genus Klebsiella has also been linked to the N2-fixation of a number of grasses, but no one of these associations has yet been shown to be symbiotic. The presence of symbiotic N2-fixing nodulation in other non-leguminous plants is also possible. Cross inoculation groups have been set up due to the limited number of host plants. An across-inoculation group is a conglomerate of leguminous species that can produce nodules in response to exposure to bacteria isolated from any nodule of that particular plant group.

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CHAPTER 6

AN ANALYSIS OF MECHANISM OF SUBSTRATE

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ABSTRACT:

Substrate level phosphorylation is when ADP is converted to ATP by the direct transfer of a phosphate group. The phosphate group is donated or transferred from a phosphorylated intermediate. This is in contrast to oxidative phosphorylation, where a chemiosmotic gradient is used to power the phosphorylation process.A simple way to think of the difference is that in substrate level, the phosphorylation is a direct process, with no middle man. The phosphate comes off of one molecule and is directly transferred to another. The energy for the phosphorylation of ADP is provided directly from breaking the high energy phosphate bond on the intermediate molecule. This is referred to as reaction coupling. During the preparatory phase, each 6-carbon glucose molecule is broken into two 3-carbon molecules. Thus, in glycolysis dephosphorylation results in the production of 4 ATP. However, the prior preparatory phase consumes 2 ATP, so the net yield in glycolysis is 2 ATP and 2 molecules of NADH are also produced and can be used in oxidative phosphorylation to generate more ATP.

KEYWORDS:

Mechanism, Substrate level,ADP, Microbiology, Microbial Physiology.

INTRODUCTION

Oxidative phosphorylation has middle men in the form of NADH and the electron transport enzymes. The phosphate comes from a pool of inorganic phosphates instead of directly from another molecule, and the energy to phosphorylate the ADP comes from the proton gradient, not from coupled reactions.The most famous examples of substrate level phosphorylation are found in the glycolysis pathway. The ATP are produced in the payoff phase of glycolysis by the action of the enzymes phosphoglycerate kinase and pyruvate kinase [1]–[3].In this unit, you will study about the mechanisms of substrate, level phosphorylation, respiratory electron transport in mitochondria and bacteria, mechanism of oxidative phosphorylation, etc.

Mechanisms Of Substrate-Level Phosphorylation

Substrate-level phosphorylation is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl group to ADP or GDP from another phosphorylated compound.Unlike oxidative phosphorylation, oxidation and phosphorylation are not coupled in the process of substrate-level phosphorylation, and reactive intermediates are most often gained in the course of oxidation processes in catabolism. Most ATP is generated by oxidative phosphorylation in aerobic or anaerobic respiration while substrate-level phosphorylation provides a quicker, less efficient source of ATP, independent of external electron acceptors. This is the case in human erythrocytes, which have no mitochondria, and in oxygen-depleted muscle.

Glycolysis

The first substrate-level phosphorylation occurs after the conversion of 3-phosphoglyceraldehyde and Pi and NAD+ to 1,3-bisphosphoglycerate via glyceraldehyde 3-phosphate dehydrogenase. 1,3-bisphosphoglycerate is then dephosphorylated via phosphoglycerate kinase, producing 3-phosphoglycerate and ATP through a substrate-level phosphorylation.The second substrate-level phosphorylation occurs by dephosphorylating phosphoenolpyruvate, catalyzed by pyruvate kinase, producing pyruvate and ATP[4]–[6].

Mitochondria

ATP can be generated by substrate-level phosphorylation in mitochondria in a pathway that is independent from the proton motive force. In the matrix there are three reactions capable of substrate-level phosphorylation, utilizing either phosphoenolpyruvate carboxykinase or succinate-CoA ligase, or monofunctional C1-tetrahydrofolate synthase.

Phosphoenolpyruvate Carboxykinase

Mitochondrial phosphoenolpyruvate carboxykinase is thought to participate in the transfer of the phosphorylation potential from the matrix to the cytosol and vice versa. However, it is strongly favored towards GTP hydrolysis, thus it is not really considered as an important source of intra-mitochondrial substrate-level phosphorylation.

Succinate-CoA Ligase

Succinate-CoA ligase is a heterodimer composed of an invariant á-sub-unit and a substrate-specific ß-sub-unit, encoded by either SUCLA2 or SUCLG2. This combination results in either an ADP-forming Succinate-CoA ligase. The ADP-forming succinate- CoAligase is potentially the only matrix enzyme generating ATP in the absence of a proton motive force, capable of maintaining matrix ATP levels under energy- limited conditions, such as transient hypoxia.

Monofunctional C1-Tetrahydrofolate Synthase

This enzyme is encoded by MTHFD1L and reversibly interconverts ADP + phosphate + -formyltetrahydrofolate to ATP + formate + tetrahydrofolate.

Other Mechanisms

In working skeletal muscles and the brain, Phosphocreatine is stored as a readily available high-energy phosphate supply, and the enzyme creatine phosphokinase transfers a phosphate from phosphocreatine to ADP to produce ATP. Then the ATP releases giving chemical energy. This is sometimes erroneously considered to be substrate-level phosphorylation, although it is a transphosphorylation.

Respiratory Electrons Transport in Mitochondria and Bacteria

The electron transport chains of bacteria operate in plasma membrane. Some bacterial electron transport chains resemble the mitochondrial electron transport chain. Paracoccus denitrificans is a gram-negative, facultative anaerobic soil bacterium.It is a model prokaryote for studies of respiration. When this bacterium grows aerobically, its electron transport chain possesses four complexes that correspond to the mitochondrial chain.But, when this bacterium grows anaerobically with nitrate as its electron acceptor, the chain is structured quite differently. Since most bacteria grow anaerobically using different variety of electron acceptor substances, the bacterial electron transport chains are frequently very different.

Bacterial electron transport chains varyin their electron carriers and are usually extensively branched. Electrons often enter at several points and leave through several terminal oxidases. Bacterial electron transport chains are usually shorter and possess lower phosphorus to oxygen ratios than mitochondrial transport chain.Thus bacterial and mitochondrial electron transport chains differ in details of construction although they operate employing the same fundamental principles. For convenience, a simplified view of the electron transport chain of Escherichia coli is being given here as to show these differences.Electron transport chain of E. Coil that operates in aerobic conditions. NADH is the electron donor. Ubiquinone is the connecting link between NADH dehydrogenase with two terminal oxidase systems of the two branches, cytochrome d branch and cytochrome o branch.

Although the electron transport chain of E. coli transports electrons from NADH to acceptors and moves protons across the plasma membrane similar to mitochondrial electron transport chain, it is quite different from the latter in its construction. E. coli transport chain is short, consists of two branches, and a quite different array of cytochromes.Coenzyme Q carries electrons and donates them to both branches, but the branches operate under different growth conditions. The cytochrome d branch shows very high affinity for oxygen and operates at low oxygen levels usually when the bacterium is in stationary phase of growth.

This branch is not as efficient as the cytochrome o branch because it does not actively pump protons to periplasmic space.The cytochrome o branch shows moderately high efficiency for oxygen and operates at high oxygen concentrations. This branch operates normally when the bacterium is in log phase of its growth, i.e., growing rapidly, and actively pumps protons in the periplasmic space.

Mechanism Of Oxidative Phosphorylation

Most of the usable energy obtained from the breakdown of carbohydrates or fats is derived by oxidative phosphorylation, which takes place within mitochondria. For example, the breakdown of glucose by glycolysis and the citric acid cycle yields a total of four molecules ofATP, ten molecules of NADH, and two molecules of FADH2. Electrons from NADH and FADH2 are then transferred to molecular oxygen, coupled to the formation of an additional to ATP molecules by oxidative phosphorylation. Electron transport and oxidative phosphorylation are critical activities of protein complexes in the inner mitochondrial membrane, which ultimately serve as the major source of cellular energy.The Electron Transport Chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called chemiosmosis. Together, the electron transport chain and chemiosmosis make up oxidative phosphorylation. The key steps of this process, shown in simplified form in the diagram above, include:

Delivery of Electrons by NADH and FADH2

Reduced electron carriers from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD–++ and FAD, which can be reused in other steps of cellular respiration.

Electron Transfer and Proton Pumping

As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H–++ ions, moving them out of the

matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.

Splitting of Oxygen to form Water

At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up H–++ to form water.

Gradient-Driven Synthesis of ATP

As H–++ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP.

The Electron Transport Chain

The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I toIV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.As the electrons travel through the chain, they go from a higher to a lower energylevel, moving from less electron-hungry to more electron-hungrymolecules. Energy is released in these 'downhill' electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the intermembrane space, forming a proton gradient.

DISCUSSION

All the components of the chain are embedded in or attached to the inner mitochondrial membrane. In the matrix, NADH deposits electrons at Complex I, turning into NAD+ and releasing a proton into the matrix. FADH2 in the matrix deposits electrons at Complex II, turning into FAD and releasing 2 H+. The electrons from Complexes I and II are passed to the small mobile carrier Q. Q transports the electrons to Complex III, which then passes them to Cytochrome C. Cytochrome C passes the electrons to Complex IV, which then passes them to oxygen in the matrix, forming water. It takes two electrons, 1/2 O2, and 2 H+ to form one water molecule. Complexes I, III, and IV use energy released as electrons move from a higher to a lower energy level to pump protons out of the matrix and into the intermembrane space, generating a proton gradient [7]–[9].

All of the electrons that enter the transport chain come from NADH and FADH2 molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.NADH is very good at donating electrons in redox reactions, so it can transfer its electrons directly to complex I, turning back into NAD–++. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the intermembrane space.FADH2 is not as good at donating electrons as NADH, so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane. Because of this 'bypass', each FADH2 molecule causes fewer protons to be pumped than an NADH.

Beyond the first two complexes, electrons from NADH and FADH2 travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called ubiquinone, which is reduced to form QH2 and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more H–++ ions are pumped across the membrane, and the electrons are ultimately delivered to another

mobile carrier called cytochrome C. Cyt C carries the electrons to complex IV, where a final batch of H–++ ions is pumped across the membrane. Complex IV passes the electrons to O2, which splits into two oxygen atoms and accepts protons from the matrix to form water. Four electrons are required to reduce each molecule of O2, and two water molecules are formed in the process.

The electron transport chain has two important functions:

Regenerates Electron Carriers

NADH and FADH2 pass their electrons to the electron transport chain, turning back into NAD–++ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.

Makes a Proton Gradient

The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H–++ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy, and, as we'll see, it can be used to make ATP.

Chemiosmosis

Complexes I, III, and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H–++ ions from the matrix to the intermembrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the proton-motive force.

Mechanism of Substrate

Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H–++ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.In the inner mitochondrial membrane, H–++ ions have just one channel available: a membrane-spanning protein known as ATP synthase. Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it's turned by the flow of H–++ ions moving down them. The electron transport chain and ATP synthase are embedded in the inner mitochondrial membrane. NADH and FADH2 made in the citric acid cycle deposit their electrons into the electron transport chain at complexes I and II, respectively.

This step regenerates NAD+ and FAD for use in the citric acid cycle. The electrons flow through the electron transport chain, causing protons to be pumped from the matrix to the intermembrane space. Eventually, the electrons are passed to oxygen, which combines with protons to form water. The proton gradient generated by proton pumping during the electron transport chain is a stored form of energy. When protons flow back down their concentration gradient, their onlyroute is throughATP synthase, an enzyme embedded in the inner mitochondrial membrane. When protons flow through ATP synthase, they cause it to turn, and its motion catalyzes the conversion of ADP and Pi to ATP.In the brown fat cells, uncoupling proteins are produced and inserted into the inner mitochondrial membrane. These proteins are simply channels that allow protons to pass from the intermembrane space to the matrix without traveling through ATP synthase. By providing an alternate route for protons to

flow back into the matrix, the uncoupling proteins allow the energy of the gradient to be dissipated as heat [10]–[12].

CONCLUSION

Substrate level phosphorylation is when ADP is converted to ATP by the direct transfer of a phosphate group. The phosphate group is donated or transferred from a phosphorylated intermediate. This is in contrast to oxidative phosphorylation, where a chemiosmotic gradient is used to power the phosphorylation process. The phosphate comes off of one molecule and is directly transferred to another. This process, in which energy from a proton gradient is used to make ATP, is called chemiosmosis. More broadly, chemiosmosis can refer to any process in which energystored in a proton gradient is used to do work. Although chemiosmosis accounts for over % of ATP made during glucose breakdown in cellular respiration, it's not unique to cellular respiration. For instance, chemiosmosis is also involved in the light reactions of photosynthesis. This might seem wasteful, but it's an important strategy for animals that need to keep warm. For instance, hibernating mammals have specialized cells known as brown fat cells.

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CHAPTER 7

A COMPREHENSIVE REVIEW OF ANAEROBIC RESPIRATION

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ABSTRACT:

The process by which the energy held in fuel is transformed into a form that a cell can utilise is known as respiration. By collecting electrons from the fuel molecule and utilising them to power an electron transport chain, the energy contained in the chemical bonds of a sugar or fat molecule is typically utilised to produce ATP. A cell must breathe to survive because without it, it would perish if it is unable to get energy from its source of fuel. Anaerobic respiration, which differs from aerobic respiration in that it does not use oxygen, is a kind of breathing. It is a different kind of respiration that is employed when there isn't any oxygen and gets its energy from neighbouring or internal substances. Anaerobic respiration is times less effective and provides less energy than aerobic respiration. To take in oxygen, most huge organisms breathe. Anaerobic cells fuel their cellular respiration using chemicals including sulphate, nitrate, sulphur, and fumarate instead of oxygen.

KEYWORDS:

Anaerobic Respiration, Microorganisms, Anaerobic cells, ATP.

INTRODUCTION

Due to its nucleus' strong affinity for electrons, molecular oxygen is the most effective electron acceptor for respiration. Certain creatures, however, have evolved to utilise different oxidizers; as a result, they can respire without oxygen. These organisms likewise employ an electron transport chain to produce as much ATP from their fuel as feasible, but since their electron acceptors are weaker than those of aerobic respiration, their electron transport chains extract less energy. Only anaerobic respiration is possible for many bacteria and archaea. Several additional creatures have the ability to breathe either aerobically or anaerobically, depending on the presence of oxygen[1]–[3].

Anesthetical Respiration

Anaerobic respiration is a different method of generating energy in which a proton motive force is produced by using an external electron acceptor other than oxygen in the electron transport chain.In contrast to aerobic respiration, which uses oxygen as an electron acceptor, anaerobic respiration uses nitrate, sulphate, carbonate, ferric ion, and even certain organic molecules as electron acceptors.These alternative electron acceptors enable microbes to breathe in an oxygen-free environment. The prokaryotes, which are anaerobic creatures, depend heavily on anaerobic respirations due to the poor solubility of oxygen in water and the great demand for oxygen as an electron acceptor by aerobic species. Hence, anaerobic respiration is exclusive to prokaryotes and uncommon in eukaryotes.

Aerobes that are Necessary and Facultative

Since they can only do anaerobic respiration and cannot utilise oxygen, the majority of bacteria are obligate anaerobes. Because of their extreme sensitivity to oxygen, it may impede or even kill them. According to what is now known, obligatory anaerobes are members of three different categories of microorganisms: a large number of prokaryotes, a few fungi, and a small number of protozoa.

The genus Clostridium is one of the most well-known groups of obligately anaerobic bacteria. It is widely distributed in soil, lake sediments, and intestinal systems and is often to blame for the spoiling of canned goods.There are several kinds of archaebacteria, methanogens, sulfate-reducing and homoacetogenic bacteria, as well as a large number of the bacteria found in mammalian guts that are obligately anaerobic.

Certain bacteria that are obligate anaerobes may tolerate oxygen and thrive in its presence even if they cannot utilise it. Aerotolerant anaerobes are those that are obligatory anaerobes.When oxygen is available in the environment, facultative aerobes engage in aerobic respiration. But, when oxygen is scarce, they convert to anaerobic respiration by using a different electron acceptor. Escherichia coli is an example of a facultative aerobe.

Metabolic Assimilative and Dissimilative Processes

In anaerobic respiration, ATP is created without the need of oxygen. While oxygen is not used as the terminal electron acceptor in this technique, the respiratory electron transport chain is still included. Instead, electron acceptors like sulphate, nitrate, or sulphur are used. As these molecules' reduction potential is smaller than that of oxygen, less energy is produced per molecule of glucose under anaerobic circumstances than under aerobic ones [4]–[6].

For anaerobic respiration, a wide range of electron acceptors may be used. Using nitrate as the last electron acceptor is denitrification. Like oxygen, nitrate has a significant potential for reduction. Several Proteobacteria employ this technique, which is widely used. Moreover, a variety of denitrifying bacteria may utilise ferric iron and organic electron acceptors.Sulfate is used as the electron acceptor in sulphate reduction, which results in the production of hydrogen sulphide as a metabolic byproduct. Several Gram-negative bacteria belonging to the -Proteobacteria employ the rather inefficient sulphate reduction pathway. Gram-positive organisms connected to Desulfotomaculum or the archaeon Archaeoglobus also utilise it.

Electron donors are needed for sulphate reduction, such as hydrogen gas or the carbon molecules lactate and pyruvate. Desulfotignum phosphitoxidans is one unique autotrophic sulfate-reducing bacterium that can utilise phosphite as an electron source.The same electron sources and acceptors employed in methanogenesis are utilised in acetogenesis, a kind of microbial metabolism, to create acetate from hydrogen and carbon dioxide.Both autotrophic and heterotrophic organisms often utilise ferric iron as a terminal electron acceptor in anaerobic reactions. Except that in ferric iron-reducing organisms, the last enzyme in this system is a ferric iron reductase, electron flow in these species is identical to that in electron transport, terminating in oxygen or nitrate. There is a lot of interest in employing certain ferric iron-reducing bacteria as bioremediation agents in ferric iron-contaminated aquifers because they may consume hazardous hydrocarbons as a carbon source.In anaerobic respiration, organic substances may also be employed as electron acceptors. Fumarate to succinate conversion, trimethylamine N-oxide to trimethylamine conversion, and dimethyl sulfoxide to dimethyl sulphide conversion are a few examples.

Reduction And Denitrification of Nitrates

Nitrate is used as an electron acceptor during denitrification, a kind of anaerobic respiration. Nitrate serves as the last electron acceptor in the respiratory electron transport chain during denitrification during anaerobic respiration. Since nitrate, like oxygen, has a high reduction potential, denitrification is a commonly employed process by many facultative anaerobes.

In a process called denitrification, nitrate is gradually converted to nitrite, nitric oxide, nitrous oxide, and other gases.The first NADH reductase, quinones, and nitrous oxide reductase transport protons across the membrane to create the electrochemical gradient necessary for respiration. Certain species can only complete the initial reduction because they only generate nitrate reductase, which results in the buildup of nitrite. Others fully cut down on nitrate. As several intermediates of complete denitrification are important greenhouse gases that combine with sunlight and ozone to form nitric acid, a component of acid rain, denitrification is a process that has an impact on the environment. Denitrification is crucial for the biological treatment of sewage because it may be utilised to limit the nitrogen released into the environment and prevent eutrophication.

Both terrestrial and marine ecosystems are subject to certain circumstances that promote denitrification. In general, it happens when oxygen levels are low and bacteria use nitrate as a terminal electron acceptor instead of oxygen to breathe. Owing to the high oxygen content of our atmosphere, denitrification can only occur under anaerobic conditions when oxygen consumption exceeds oxygen supply and nitrate concentrations are adequate. Some examples of these habitats are specific soils and groundwater, wetlands, oil reserves, poorly ventilated ocean nooks, and sediments on the sea bottom.

The nitrogen cycle and the function of soil microorganisms the process of denitrification is crucial to the upkeep of ecosystems. In general, denitrification occurs in low-oxygen settings.While autotrophic denitrifiers have also been found, heterotrophic bacteria are predominantly responsible for denitrification. It has been discovered that more than one enzyme route is involved in the full reduction of nitrate to molecular nitrogen, which often involves many bacterial species.

Rhizobia are soil microorganisms that have the unusual capacity to create a N2-fixing symbiosis on the roots of legume plants. Certain Rhizobia species have the ability to transition from O2-respiration to nitrate-respiration in the event of a lack of oxygen.Organisms with the nrf- gene are capable of directly reducing nitrate to ammonium. In most ecosystems, denitrification is more frequent than this technique of nitrate reduction. Alcaligenes faecalis, Alcaligenes xylosoxidans, Pseudomonas spp., Bradyrhizobium japonicum, and Blastobacter denitrificans are only a few examples of the organisms that have the nir and nos genes, which are also involved in denitrification.

Reduction of Sulflate and Sulphur

Sulfate is used as a terminal electron acceptor in the electron transport chain during sulphate reduction, a kind of anaerobic respiration. Sulfate is used as a terminal electron acceptor in the electron transport chain during sulphate reduction, a kind of anaerobic respiration. Sulfate reduction is a very inefficient process when compared to aerobic respiration, yet it is an essential mechanism for bacteria and archaea living in oxygen-poor, sulfate-rich environments.Although some sulphate reducers are lithotrophic and utilise hydrogen gas as an electron donor, others are organotrophic and employ carbon molecules like lactate and pyruvate as electron donors. Although certain unique autotrophic sulfate-reducing bacteria are capable of sulphur disproportionation, employing elemental sulphur, sulfite, and

thiosulfate to make both hydrogen sulphide and sulphate, others may utilise phosphite as an electron donor.

Sulfate has to be activated before it can be utilised as an electron acceptor. The ATP-sulfurylase enzyme does this by converting sulphate and ATP into adenosine - phosphosulfate. After that, APS is broken down into sulfite and AMP. Sulfite is subsequently further reduced to sulphide, and an additional molecule of ATP is used to convert AMP into ADP. In order to complete the process, two molecules of the energy carrier ATP must be used up and then recovered by reduction.Strict anaerobes are all organisms that reduce sulphate. Sulfate must first be activated by adenylation to generate APS in order to be metabolised, which uses ATP since it is energetically stable. The enzyme APS reductase subsequently breaks down the APS to produce sulfite and AMP. The fermentation of the carbon substrate accounts for the ATP used by organisms that employ carbon molecules as electron donors. Indeed, respiration during sulphate reduction is propelled by the hydrogen created during fermentation.

As they began contributing to the sulphur cycle not long after life first appeared on Earth, sulfate-reducing bacteria are among the earliest types of microbes, dating back to 3.5 billion years ago. Sulfate-reducing microorganisms are widespread in anaerobic settings and help in the breakdown of organic molecules. Fermenting bacteria in these anaerobic settings break down big organic molecules for energy; the smaller chemicals that result are then further oxidised by acetogens, methanogens, and the rival sulfate-reducing bacteria.Assimilatory sulphate reduction is a common process used by many bacteria to convert modest quantities of sulphates into cell components that include sulphur. Dissimilatory sulphate reduction is the process by which sulfate-reducing bacteria reduce sulphate in vast quantities to produce energy and expel the resultant sulphide as trash. The majority of sulfate-reducing bacteria are also capable of reducing sulfite, thiosulfate, or elemental sulphur.

Sulfate-reducing bacteria produce toxic hydrogen sulphide as one of their waste products, and its rotten egg stench is often used to identify their presence in natural environments. Salt marshes and mud flats emit sulphurous scents as a result of sulfate-reducing microorganisms. Metal sulphides will be created when a large portion of the hydrogen sulphide reacts with the metal ions in the water. These insoluble metal sulphides, such ferrous sulphide, are often black or brown and contribute to the sludge's dark appearance. Consequently, metal sulphides produced by sulfate-reducing bacteria are the cause of the black hue of pond sludge.

Black Sludge

Metal sulphides, produced by the activity of sulfate-reducing bacteria, are responsible for the pond's dark hue.The sulphate in marine sediments is also thought to be significantly sunk by this mechanism. Biocide chemicals are often added to water in hydrofracturing fluids to limit the microbial activity of sulfate-reducing bacteria in order to prevent anaerobic methane oxidation and to reduce possible production loss. These fluids are used to frack shale deposits to extract methane.Metal constructions exposed to sulfate-containing water often experience issues brought on by sulfate-reducing microorganisms. A layer of molecular hydrogen is formed on the metal surface as a result of the interaction between water and metal. This hydrogen is oxidised by sulfate-reducing bacteria, which produces hydrogen sulphide and aids in corrosion. Also contributing to the biogenic sulphide corrosion of concrete and tainting crude oil is hydrogen sulphide produced by sulfate-reducing bacteria.Certain species of sulfate-reducing bacteria, including those that can break down hydrocarbons like benzene, toluene, ethylbenzene, and xylene, may be used to clean up polluted soils. Acid mine fluids could also be handled by sulfate-reducing microorganisms.

Respirative Carbonation

In order to save energy during anaerobic respiration, two main categories of obligate anaerobic prokaryotes employ CO2 as an electron acceptor and hydrogen as a significant electron donor. A summary of the processes of acetogenesis and methanogenesis, which are carried out by homoacetogens and methanogens, respectively.Ion gradients of H+ or Na+ are produced by acetogenesis, while methanogenesis produces H+ ion gradients during electron transport. Through ATPase, these forces propel the production of ATP. By substrate-level phosphorylation, ATP production is also a part of acetogenesis.

Acetogenesis

Homoacetogens Carry Out Acetogenesis

One molecule of CO2 is reduced to the methyl group of acetate, while the other molecule is reduced to the carbonyl group of acetate. These two linear routes are then combined at the end to generate Acetyl-CoA, which is subsequently transformed to acetate in these bacteria.When a sodium gradient is generated, creating sodium motive force across the plasma membrane, the ATP is produced in the Acetyl-CoA pathway of CO2 reduction to acetate. A Na+-powered ATPase enzyme may produce ATP when the plasma membrane is in an active condition. When acetyl-CoA is converted to acetate through acetyl-phosphate, additional ATP is also produced via substrate-level phosphorylation.

Methanogenesis

Methanogenesis, also known as biomethanation, is a kind of anaerobic respiration that produces methane by using carbon as the terminal electron acceptor. A few low molecular weight organic molecules, including carbon dioxide, acetic acid, formic acid, methanol, methylamines, dimethyl sulphide, and methanethiol, are the source of the carbon. Acetic acid or carbon dioxide are used as the terminal electron acceptor in the two methanogenesis routes that have been most thoroughly studied:

Acetate Methanogenesis

Methanogenesis, the process by which acetate is converted to methane, is carried out by a class of microbes known as methanogens. While many of them coexist closely with anaerobic bacteria, they have only been found in the domain Archaea, which is phylogenetically separate from eukaryotes and bacteria. In most situations, the degradation of biomass is completed by the formation of methane, which is a significant and common type of microbial metabolism.Electron acceptors are depleted throughout the decay process, while fermentation-produced hydrogen, carbon dioxide, and light organics build up. Apart for carbon dioxide, which is a byproduct of the majority of catabolic processes, all electron acceptors become depleted at advanced phases of organic breakdown. Unlike other potential electron acceptors, it is not depleted.

DISCUSSION

In the absence of any other electron acceptors than carbon, only methanogenesis and fermentation may take place. Only bigger organic molecules can be broken down during fermentation, which results in the production of tiny organic compounds. The semi-final byproducts of decay, including hydrogen, tiny organics, and carbon dioxide, are efficiently removed by methanogenesis. Without methanogenesis, anaerobic areas would see significant carbon buildup [7]–[9].Methanogenesis also takes place in the digestive systems of people and other animals, particularly ruminants. Anaerobic organisms, such as methanogens, break

down cellulose in the rumen into forms the animal can consume. Cattle and other animals like them wouldn't be able to eat grass without the help of these microbes. The gut is where the beneficial byproducts of methanogenesis are absorbed. Mostly by belching, the animal exhales methane. A typical cow produces 0 litres of methane every day. Some people, but not all, release methane via their flatulence!

Although other studies show that plants do not truly produce methane; rather, they just take it from the soil and subsequently leak it via their leaf tissues, other trials have even shown that the leaf tissues of live plants emit methane. While it is far from clear, there may yet be some undiscovered process by which plants create methane.

CONCLUSION

The process by which the energy stored in fuel is transformed into a form that a cell can utilise is known as respiration. By collecting electrons from the fuel molecule and utilising them to power an electron transport chain, the energy contained in the chemical bonds of a sugar or fat molecule is typically utilised to produce ATP. A cell must breathe to survive because without it, it would perish if it is unable to get energy from its source of fuel. Anaerobic respiration, which differs from aerobic respiration in that it does not use oxygen, is a kind of breathing. With a potential to cause global warming that is times larger than carbon dioxide, methane is one of the planet's most significant greenhouse gases. As a result, methane generated by the methanogenesis process in animals plays a significant role in global warming. Methanogenesis may also be used profitably. It may be used to remediate organic waste and to create valuable chemicals. It is the main mechanism through which organic matter decomposes in landfills. Methane produced by biological processes may be captured and utilised as a sustainable fuel substitute.

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CHAPTER 8

BACTERIAL TRANSPORT AND QUORUM SENSING

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ABSTRACT:

Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate. Bioenergetic processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism, therefore to life itself. This energy is used to drive chemical reactions and help store and process information, which is essential in propagating life. Energy may be obtained from sunlight, in which case the organisms are referred to as phototrophs, or it may be extracted from chemicals, in which case the organisms are referred to as chemotrophs. Because energy may not be available at all times to fuel these life processes, organisms have adapted mechanisms to couple chemical reactions so that exergonic reactions can provide energy for those that are endorgernic. Environmental materials that organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms. The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water together.

KEYWORDS:

Bacterial Transport, Bacterial Growth,Microorganisms, Bioenergetics, Microbial Physiology.

INTRODUCTION

The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen. The oxidation releases energy because stronger bonds have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival [1]–[3].Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular processes such as cellular respiration and the many other metabolic and enzymatic processes that lead to production and utilization of energy in forms such as adenosine triphosphate molecules. That is, the goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics. Bioenergetics is the part of biochemistryconcerned with the energyinvolved in making and breaking of chemical bonds in the molecules found in biological organisms. It can also be defined as the study of energy relationships and energy transformations and transductions in living organisms.

The ability to harness energy from a varietyof metabolic pathways is a property of all living organisms. Growth, development, anabolism and catabolism are some of the central processes in the study of biological organisms, because the role of energy is fundamental to such biological processes. Life is dependent on energytransformations; living organisms

survive because of exchange of energybetween living tissues/ cells and the outside environment. Some organisms, such as autotrophs, can acquire energyfrom sunlight without needing to consume nutrients and break them down. Other organisms,like heterotrophs, must intake nutrients from food to be able to sustain energy by breaking down chemical bonds in nutrients during metabolic processes such as glycolysis and the citric acid cycle. Importantly, as a direct consequence of the First Law of Thermodynamics, autotrophs and heterotrophs participate in a universal metabolic networkby eating autotrophs, heterotrophs harness energy that was initially transformed by the plants during photosynthesis.

In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work or for other processes, when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy.Adenosine triphosphate is the main 'energy currency' for organisms; the goal of metabolic and catabolic processes are to synthesize ATP from available starting materials, and to break- down ATP into adenosine diphosphate and inorganic phosphate by utilizing it in biological processes. In a cell, the ratio of ATP to ADP concentrations is known as the 'energy charge' of the cell. Acell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation.

Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed to adenosine diphosphate and inorganic phosphate. Here it is the thermodynamicallyfavorable free energyof hydrolysis that results in energyrelease; the phosphoanhydride bond between the terminal phosphate group and the rest of the ATP molecule does not itself contain this energy. An organism's stockpile of ATP is used as a battery to store energy in cells. Utilization of chemical energy from such molecular bond rearrangement powers biological processes in every biological organism.

Living organisms obtain energy from organic and inorganic materials, i.e. ATP can be synthesized from a variety of biochemical precursors. For example, lithotrophs can oxidize minerals such as nitrates or forms of sulfur, such as elemental sulfur, sulfites, and hydrogen sulfide to produce ATP. In photosynthesis, autotrophs produce ATP using light energy, whereas heterotrophs must consume organic compounds, mostly including carbohydrates, fats, and proteins. The amount of energy actually obtained by the organism is lower than the amount present in the food; there are losses in digestion, metabolism, and thermogenesis.

Environmental materials that organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms. The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water together. The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen. The oxidation releases energy because stronger bonds have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival [4]–[6].

Free energy is the useful energy also known as the chemical potential. The first law of thermodynamics states that 'the total energyof a system plus its surroundings remains constant'. This is also the laws of conservation of energy. Energy may be transferred from one part to another or may be transformed into an-other form of energy. The second law of

thermodynamics states that 'the total entropy of a system must increase if a process is to occur spontaneously'.

Entropy

Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium. Under constant temperature and pressure, the relationship between the free energy change and the change in entropy is given by the following equation which combines the two laws of thermodynamics.

Enthalpy

In a process carried out at constant volume, the heat content of a system is equal to internal energy, as no PV work is done. But, in a constant pressure process, the system also expends energy in doing PV work.The reactions which are accompanied by release of heat energy are called as exothermic reactions. In such cases, there is negative change in enthalpy from reactants to products. For example, in combustion of glucose to CO2 + H2O, large amount of heat is released. Therefore, this is an exothermic reaction with -□H. melting of ice into liquid water and its subsequent vaporization into water vapours absorb considerable heat from the surroundings, therefore this is an endothermic reaction with + ΔH.

Electron Carriers

Electron carriers are important molecules in biological systems. They accept electrons and move them as part of the electron transport chain, transferring the electron, and the energy it represents, to power the cell. Electron carriers are vital parts of cellular respiration and photosynthesis. In each reaction, when a molecule donates an electron, it is said to be oxidized and the electron acceptor is said to be reduced. Oxidation-reduction reactions always happen in matched pairs; no molecule can be oxidized unless another is reduced. It is often bound firmly to an enzyme site, so not very much free FAD exists in a cell. Some FAD is formed during the citric acid cycle of cellular respiration. One function of FAD is to donate electrons to oxidative phosphorylation, where the electrons from the reduced form of FAD are transferred to create adenosine triphosphate, and the energy currency of the cell. This process happens in the mitochondria of the cell.

Nicotinamide Adenine Dinucleotide, abbreviated NAD, nicotinamide adenine dinucleotide consists of two ribose sugar rings attached to each other with phosphate groups, with an adenine unit on one side and a nicotinamide ring on the other. The nicotinamide ring is the active part of the molecule, accepting and donating two electrons. NAD acts as an electron acceptor during glycolysis and the citric acid cycle of cellular respiration and donates them to oxidative phosphorylation. The closely related nicotinamide adenine dinucleotide phosphate is produced in the light reactions of photosynthesis and consumed in the Calvin cycle.Coenzyme Q also known as ubiquinone, has ten repeating isoprene units. This makes it soluble in fats rather than water, meaning it is valuable in moving electrons across the phospholipid mitochondrial membranes. Like FAD, coenzyme Q can accept one or two electrons. Coenzyme Q is integral to the first three steps of the electron transport chain, moving electrons across the inner mitochondrial membrane as part of oxidative phosphorylation.

Cytochrome C is important at the end of the electron transport chain in the mitochondria, or in bacterial cells, which do not have mitochondria. Cytochromes are proteins forming a matrix around an iron atom, known as the heme group. The heme group accepts electrons one at a time until it has four stored in the protein matrix. Cytochrome C is a free molecule, which

allows it to move electrons between the larger and less mobile electron carrier molecules. Cytochrome C transports electrons between the third and final steps of the electron transport chain.

Artificial Electron Donors

The use of artificial electron donors has been one of the biochemical approaches to the elucidation of complex electron transport chains in biological membranes. In photosynthesis it started with Hill's discovery that ferric salts could induce light- dependent oxygen evolution in cell-free leaf extracts. Since then the results of this approach has taught about the sequence, about the energy-conserving steps, and more recently about the topography of the photosynthetic electron transport chain in the chloroplast membrane. It has also made possible the design of assays for parts of the chain in a physiologically nonfunctional state, i.e. for the reaction center complexes, either during biochemical isolation or during biogenesis of the photosynthetic apparatus.

Inhibitors

An inhibitor is any agent that interferes with the activity of an enzyme. Inhibitors may affect the binding of enzyme to substrate, or catalysis, or both. Researchers use enzyme inhibitors to define metabolic pathways and to understand enzyme reaction mechanisms. Manydrugs are designed as inhibitors of target enzymes. Inhibition is also a natural phenomenon. Cells regulate metabolic pathways by specific inhibition of key enzymes.Enzyme inhibitors are divided into two classes, irreversible and reversible. Irreversible inhibition implies destruction or permanent modification of chemical groups in the enzyme. In contrast, reversible inhibitors form a complex with the enzyme that can dissociate and release the active enzyme. An enzyme E can bind either to substrate S, to form an ES complex or to inhibitor I, to form the complex EI.There are two major types of reversible inhibitors, competitive and non- competitive. Competitive inhibition can be reversed by increasing substrate concentration, whereas noncompetitive inhibition cannot be reversed by adding more substrate.

DISCUSSION

The classic example of competitive inhibition is inhibition of succinate dehydrogenase, an enzyme, by the compound malonate. Hans Krebs first elucidated the details of the citric acid cycle by adding malonate to minced pigeon muscle tissue and observing which intermediates accumulated after incubation of the mixture with various substrates. The structure of malonate is very similar to that of succinate. The enzyme will bind malonate but cannot act further on it. That is, the enzyme and inhibitor form a nonproductive complex. We call this competitive inhibition, as succinate and malonate appear to compete for the same site on the enzyme. With competitive inhibition, the percent of inhibition is a function of the ratio between inhibitor and substrate, not the absolute concentration of inhibitor [7]–[9].

Noncompetitive inhibition is not reversed by adding more substrate. The inhibitor binds at a site on the enzyme other than the substrate binding site. The inhibitor can bind to either free enzyme, forming EI, or to the ES complex, forming EIS. Both EI and EIS are nonproductive.Irreversible inhibitors are those that permanently disable the enzyme. The complex EI or EIS cannot dissociate, so that these are dead-end complexes. When an irreversible inhibitor is added to the enzyme-containing solution, inhibition may not be complete immediately, but increases gradually with time, as more and more enzyme molecules are modified.

Uses of Inhibitors

Among the irreversible inhibitors are organophosphorus compounds, which inhibit the enzyme acetylcholinesterase and similar enzymes. Organophosphorous compounds include nerve gases that work on the human nervous system, and insecticides like Malathion.Many therapeutic drugs are enzyme inhibitors. Important examples are penicillin, which inhibits an enzyme necessaryfor bacterial cell wall synthesis, and aspirin, an inhibitor of the synthesis of molecules that mediate pain and swelling. More recent examples are drugs used in the treatment of human immunodeficiency virus and acquired immunodeficiency syndrome that prevent maturation of the virus by inhibiting the HIV protease, and drugs that lower cholesterol by inhibiting a key step in cholesterol biosynthesis.

Uncouplers

Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain andATP synthase -ATPase). Miscellaneous compounds are known to be uncouplers, but weakly acidic uncouplers are representative because they show very potent activities. The most potent uncouplers discovered so far are the hindered phenol SF, and hydrophobic salicylanilide S-, which are active in-vitro at concentrations in the nm range. For induction of uncoupling, an acid dissociable group, bulky hydrophobic moiety and strong electron-withdrawing group are required. Weakly acidic uncouplers are considered to produce uncoupling by their protonophoric action in the H -impermeable mitochondrial membrane. Forexertingthese effects, the stability of the respective uncoupler anions in the hydrophobic membrane is veryimportant. High stability is achieved bydelocalization of the polar ionic charge through uncoupler -specific mechanisms. Such an action of weaklyacidic uncouplers is characteristic of the highly efficient membrane targeting action of a nonsite-specific type of bioactive compound.

Phosphorylation

Certain organic phosphates, formed in the tissues out of anabolic chemical reaction, play a key role in the energy transactions of living organisms. The importance of organic phosphates in metabolism was first indicated by the studies of Harden and Young, who found that fermentation of glucose by cell-free yeast juice in-creased rapidly when inorganic phosphorus is added to the medium, which was later converted to organically bound phosphate.

The work of Embden, Meyerhof, Cori and their associates show that organic phosphates concerned with the metabolism of glucose by yeast are also intermediates in the metabolism of glycogen and glucose in muscle and other tissues.The presence ofATP, ADP, CPand other high-energy phosphate compounds in the tissue and their participation in different metabolic processes indicate the importance of organic phosphates in metabolism. The sugar phosphates, although not high-energycompounds, are obligatory intermediates. Their formation involves phosphorylation bythe high-energy compound ATP in presence of suitable enzymes.The term phosphorylation includes all chemical reactions in the body which require combination with phosphoric acid. The reverse changes are called dephosphorylation, where phosphoric acid is dissociated from the compound.These two processes are probably the reversible reactions of the same enzyme system.

1. Physiological Importance of Phosphorylation

Phosphoric acid enters into the composition of cell protoplasm. Hence, phosphorylation is an essential chemical process for all cells. In addition to this it takes an essential part during absorption and metabolism of different foodstuffs.

2. Oxidative Phosphorylation

This reaction can take place only in presence of intact oxidation process and the energy-yielding mechanism is coupled with energy-harnessing mechanism. Uncoupling agents like 2, 4-dinitrophenol inhibit phosphorylation but not oxidation. They elevate the oxygen consumption rate.

i. Enzymes

The enzymes that take part in this process are phosphorylase, phosphatase, etc.

ii. Hormones

- Adrenal cortex is believed to be directly responsible for phosphorylation. Glucocorticoids inhibit phosphorylation adrenalectomy accelerate it.

- Anterior pituitary, byits adrenocorticotrophic hormone, mayexert a superior control on phosphorylation, through adrenal cortex. The marasmic condition that develops in diseases of adrenal cortex may be partly explained by the consequent disturbance of phosphorylation, which affects absorption, metabolism and nutrition of the body. Growth hormone affects phosphorylation in a similar way as glucocorticoids.

iii. Inorganic Salts

Sodium may have some effects on phosphorylation.It is believed that adrenal cortex controls phosphorylation by helping the enzymes responsible for the process. But it is also known that many of the defects, seen in diseases of the adrenal cortex, are rectified by giving enough NaCl. For instance, the defective fat absorption in adrenalectomised animals improves by giving NaCl.

It is interesting to note that it is the same adrenal cortex which also controls Na metabolism. From these observations it may be suggested that adrenal cortex controls phosphorylation not by a direct action on the enzymes but by an indirect influence on Na metabolism.It is also known that phospholipids are antagonistic to cholesterol. These two compounds are always found to remain together. It is also interesting to note that adrenal cortex which controls phosphorylation and therefore phospholipid formation controls metabolism of sterols.

Bond Energy

Bond energy is defined as the amount of energy required to break apart a mole of molecules into its component atoms. It is a measure of the strength of a chemical bond. Bond energy is also known as bond enthalpy or simply as bond strength.Bond energy is based on an average of bond dissociation values for species in the gas phase, typically at a temperature of 8 Kelvin[10]–[12].

CONCLUSION

Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate. Bioenergetic processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism,

therefore to life itself. This energy is used to drive chemical reactions and help store and process information, which is essential in propagating life. Energy may be obtained from sunlight, in which case the organisms are referred to as phototrophs, or it may be extracted from chemicals, in which case the organisms are referred to as chemotrophs. It may be found by measuring or calculating the enthalpy change of breaking a molecule into its component atoms and ions and dividing the value by the number of chemical bonds. For example, the enthalpy changes of breaking methane into a carbon atom and four hydrogen ions, divided by four bonds, yields the bond energy. Bond energyis not the same thing as bond-dissociation energy. Bond energy values are an average of the bond-dissociation energies within a molecule. Breaking subsequent bonds requires a different amount of energy.

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CHAPTER 9

AN ANALYSIS OF TRANSPORT ACROSS MEMBRANE

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ABSTRACT:

In cellular biology, membrane transport refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them. The regulation of passage through the membrane is due to selective membrane permeability a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. In other words, they can be permeable to certain substances but not to others. The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules. As the diversityand physiology of the distinct cells is highlyrelated to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage.

KEYWORDS:

Transport, Bacterial Growth,Membrane, Proteins, Microbial Physiology.

INTRODUCTION

This differential expression is regulated through the differential transcription of the genes coding for these proteins and its translation, for instance, through genetic-molecular mechanisms, but also at the cell biology level: the production of these proteins can be activated by cellular signaling pathways, at the biochemical level, or even by being situated in cytoplasmic vesicles [1]–[3].In this unit, you will study about how transport across membrane occurs, diffusion, osmosis, active transport and group translocation.

Transport across Membrane

Uptake of the required nutrients by the microbial cell is important. Since microorganisms live in nutrient poor habitats, theymust be able to transport nutrients from dilute solutions into the cell against concentration gradient. Finally, they must pass through a selectively permeable plasma membrane. Microorganisms use different transport mechanisms like facilitated diffusion, active transport and group translocation. Eukaryotic microorganisms do not employ group translocation but take up nutrients by endocytosis.Movement of materials across the plasma membrane is mostly done by two processes.

Passive Process

Substances cross the area from an area of high concentration to an area of low concentration without any expenditure of energy. For example, simple diffusion, osmosis and facilitated diffusion.

Active Process

The cell must use energy to move substances from areas of low concentration to areas of high concentration. For example, group translocation.

Passive Processes

Passive or Simple Diffusion

Often called diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration. The rate is dependent on the size of the concentration gradient between a cell's exterior and its interior. Very small molecules such as water and oxygen and carbon dioxide move across membranes by simple or passive diffusion. Larger molecules, ions, and polar substances do not cross membranes by this method.

Osmosis

It is the net movement of solvent molecules across a selectively permeable membrane from an area in which the solvent molecules are highly concentrated to an area of low concentration until equilibrium is reached. In living systems, the chief solvent is water. The three types of solutions which are normally found are isotonic, hypotonic and hypertonic.

Facilitated Diffusion

The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called as facilitated diffusion. Carrier proteins also resemble enzymes in their specificity for the substances to be transported; each carrier is selective and will transport onlycloselyrelated solutes. Because there is no energyinput, molecules will continue to enter onlyas long as their concentration is greater on the outside. Two widespread major intrinsic protein channels in bacteria are aquaporins that transport water and glycerol facilitators which aid glycerol diffusion. The carrier protein complex spans the membrane. After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier would subsequently change back to its original shape and be ready to pick up another molecule. The mechanism is driven by concentration gradients and therefore is reversible [4]–[6].For example, Glycerol is transported by facilitated diffusion in E. coli, Salmonella typhimurium, Pseudomonas, Bacillus and many other bacteria. This is prominent in eukaryotes where it is used to transport a variety of sugars and amino acids.

Active Transport

Active transport is the transport of solute molecules to higher concentrations or against a concentration gradient, with the use of metabolic energyinput. It resembles facilitated diffusion in the involvement of protein carrier activity, but differs in its use of metabolic energy and in its ability to concentrate substances. One example of active transport is binding protein transport systems or ATP-binding cassette transporters are active in bacteria, archaea and eukaryotes.

ABC Transporters

These transporters consist of two hydrophobic membrane spanning domains associated on their cytoplasmic surfaces with two nucleotide-binding domains. The membrane spanning domains from a pore in the membrane and the nucleotide binding domains bind and

hydrolyzeATP to drive uptake.ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of the gram positive plasma membrane. These binding proteins, bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecule inside the cell. E. coli transports a variety of sugars and amino acids by this mechanism.Eukaryotic ABC transporters are sometimes of great medical importance. Some tumor cells pump drugs out using these transporters. Cystic fibrosis results from a mutation that inactivates an ABC transporter that acts as a chloride ion channel in lungs.

Bacteria also use Proton gradients generated during electron transport to drive active transport. The lactose permease of E. coli transports a lactose molecule inward as a proton simultaneously enters the cell. Such linked transport of two substances in the same direction is called Symport. E. coli also uses proton symport to take up amino acids and organic acids like succinate and malate.Aproton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient. In E. coli, sodium transport system pumps sodium outward in response to the inward movement of protons. Such linked transport in which the transported substances move in opposite directions is called Antiport. The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids. E.coli has at least transport systems for the sugar galactose.

Group Translocation

In active transport, solute molecules move across a membrane without modification. Many prokaryotes also take up molecules by group translocation, a process in which a molecule is transported into the cell while being chemically altered. For example, Phosphoenolpyruvate: Sugar phosphotransferease system. It transports a variety of sugars while phosphorylating them using phosphoenolpyruvate as the phosphate donor.In E. coli and Salmonella typhimurium, it consists of two enzymes and a low molecular weight heat stable protein. HPR and enzyme I are cytoplasmic. Enzyme II is more variable in structure and often composed of three sub units or domains. EIIA is cytoplasmic and soluble. EIIB also is hydrophilic but frequently attached to EIIC, a hydrophobic protein that is embedded in the membrane. A high energy phosphate is transferred from PEP to enzyme II. PTS's are widely distributed in prokaryotes. Aerobic bacteria lack PTS's. Genera Escherichia, Salmonella, Staphylococcus and other facultative anaerobic bacteria have phosphotransferase systems; some obligate anaerobic bacteria also have PTS's. Many carbohydrates are transported by these systems. E. coli takes up glucose, fructose, mannitol, sucrose, N-acetylglucosamine, cellobiose and other carbohydrates by group translocation.

Iron Uptake

All microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric ion and its derivatives, which leave little free, iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores. Siderophores – are low molecular weight molecules that are able to complex with ferric ion and supply it to the cell. These are either hydroxamates or phenolates-catecholates. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by E.coli. Microorganisms secrete siderophores when little iron is available in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore receptor protein.

DISCUSSION

The iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. In E.coli, the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After the iron has entered the cell, it is reduced to the ferrous form. Iron is so crucial to microorganisms that many use more than one route of iron uptake to ensure an adequate supply.

Uptake of the required nutrients by the microbial cell is important. Since microorganisms live in nutrient poor habitats, they must be able to transport nutrients from dilute solutions into the cell against concentration gradient. Finally, they must pass through a selectively permeable plasma membrane. Microorganisms use different transport mechanisms like facilitated diffusion, active transport and group translocation. Eukaryotic microorganisms do not employ group translocation but take up nutrients by endocytosis. The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called permeases which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called as facilitated diffusion. Carrier proteins also resemble enzymes in their specificity for the substances to be transported; each carrier is selective and will transport only closely related solutes [7]–[9].

As there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside. Two widespread major intrinsic protein channels in bacteria are aquaporins that transport water and glycerol facilitators which aid glycerol diffusion. Active transport is the transport of solute molecules to higher concentrations or against a concentration gradient, with the use of metabolic energy input. It resembles facilitated diffusion in the involvement of protein carrier activity, but differs in its use of metabolic energy and in its ability to concentrate substances.

ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of the gram positive plasma membrane. A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient. All microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric ion and its derivatives, which leave little free, iron available for transport. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by E.coli.

Microorganisms secrete siderophores when little iron is available in the medium. The iron is either released to enter the cell directly or the whole iron- siderophore complex is transported inside by an ABC transporter.In E.coli, the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter [10]–[12].

CONCLUSION

In cellular biology, membrane transport refers to thecollection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them. The regulation of passage through the membrane is due to selective membrane permeability - a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules. As

the diversity and physiology of the distinct cells is highly related to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage. This differential expression is regulated through the differential transcription of the genes coding for these proteins and its translation, for instance, through genetic-molecular mechanisms, but also at the cell biology level: the production of these proteins can be activated by cellular signaling pathways, at the biochemical level, or even by being situated in cytoplasmic vesicles.

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CHAPTER 10

ENERGY, ENVIRONMENT AND MICROBIAL SURVIVAL: A REVIEW

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ABSTRACT:

The biosphere has been shaped both by physical events and by inter- actions with the organisms that occupy it. Among living organisms, prokaryotes are much more metabolically diverse than eukaryotes and can also thrive under a variety of extreme conditions where eukaryotes cannot. This is possible because of the wealth of genes, metabolic pathways and molecular processes that are unique to prokaryotic cells. For this reason, prokaryotes are very important in the cycling of elements, including carbon, nitrogen, sulfur and phos- phorus, as well as metals and metalloids such as copper, mercury, selenium, arsenic and chromium. After publication in of the first full DNA sequence of a free- living bacterium, Haemophilus influenzae, whole genome sequences of hundreds of prokaryotes have now been determined and many others are currently being sequenced. Our knowledge of the whole genome profoundly influences all aspects of microbiology. Determination of entire genome sequences, however, is only a first step in fully understanding the properties of an organism and the environment in which the organism lives. The functions encoded by these sequences need to be elucidated to give biochemical, physiological and ecological meaning to the informa- tion. Furthermore, sequence analysis indicates that the biological functions of substantial portions of complete genomes are so far unknown.

KEYWORDS:

Energy, Bacterial Growth,Environment, Microbial, Microbial Physiology.

INTRODUCTION

A full understanding of the com- plex biological phenomena that occur in the biosphere therefore requires a deep knowledge of the unique biological processes that occur in this vast prokaryotic world.Defining the role of each gene in the complex cellular metabolic network is a formidable task. In addition, genomes contain hundreds to thousands of genes, many of which encode multiple proteins that interact and function together as multicomponent systems for accomplishing specific cellular processes. The products of many genes are often co-regulated in complex signal transduction networks, and understanding how the genome functions as a whole presents an even greater challenge. It is also known that for a sig- nificant proportion of metabolic activities, no representative genes have been identified across all organisms, such activities being termed ‘orphan’ to indicate they are not currently assigned to any gene. This also represents a major future challenge and will require both computational and experimental approaches [1]–[3].

It is widely accepted that less than 1% of prokaryotes have been cultivated in pure culture under laboratory conditions. Development of new sequencing techniques has allowed us to obtain genomic information from the multitudes of unculturable prokaryotic species and

complex microbial populations that exist in nature. Such infor- mation might provide a basis for the development of new cultivation techniques. Elucidation of the function of unknown genes through a better understanding of biochemistry and physiology could ulti- mately result in a fuller understanding of the complex biological phenomena occurring in the biosphere.

Unlike multicellular eukaryotes, individual cells of unicellular prokaryotes are more exposed to the continuously changing environment, and have evolved unique structures to survive under such conditions. Life can be defined as a reproduction process using materials available from the environment according to the genetic information possessed by the organism. Utilization of the materials available in the environment necessitates transport into cells that are separated from the environment by a membrane.

Many prokaryotes, including Escherichia coli, can grow in a simple mineral salts medium containing glucose as the sole organic com- pound. Glucose is metabolized through glycolytic pathways and the tricarboxylic acid cycle, supplying all carbon skeletons, energy in the form of ATP and reducing equivalents in the form of NADPH for growth and reproduction.As mentioned repeatedly in this book, the goal of life is preservation of the species through reproduction, but this requires energy. Although there are a few exceptional copiotrophic environments such as foodstuffs and animal guts, most ecosystems where micro- organisms are found are oligotrophic. Those organisms that can utilize nutrients efficiently have a better chance of survival in such ecosystems. Further, many microbes synthesize reserve materials, when available nutrients are in excess, and utilize these under star- vation conditions, while various resting cells are produced under conditions where growth is difficult. In this chapter, the main bacter- ial survival mechanisms are discussed in terms of reserve materials and resting cell types [4]–[6].

As discussed earlier, living microorganisms maintain a certain level of adenylate energy charge and proton motive force even under starvation conditions these forms of biological energy are needed for the basic metabolic processes necessary to survive such as transport and the turnover of macromolecules. Maintenance energy is the term used for this energy.Under starvation conditions, cells utilize cellular components including reserve and non-essential materials for survival. This is referred to as endogenous metabolism. Almost all prokaryotes accu- mulate at least one type of reserve material under energy-rich condi- tions. During a period of starvation, the reserve material are consumed through endogenous metabolism before the organism oxidizes other cellular constituents such as proteins and RNA that are not needed under the starvation conditions. When a population starves, some individuals die thereby providing an energy source for other members of the population in a mechanism known as programmed cell death.

RNA and proteins used for endogenous metabolism are not referred to as reserve materials since they have specific functions other than as substrates for endogenous metabolism. Reserve material can be defined as polymers synthesized when an energy source is supplied in excess, and used as a substrate for endogenous metabolism with- out any other cellular functions. Almost all prokaryotes accumulate at least one type of reserve material. It is likely that the ability to accumulate reserve materials is advantageous for survival in natural habitats.Reserve materials in bacteria can be grouped into four categories according to their chemical nature. These are carbohydrates such as glycogen, lipids such as poly-β-hydroxybutyrate, polypeptides and polyphosphate.

Glycogen is utilized by the action of two enzymes, a debranching enzyme and glycogen phosphorylase. Escherichia coli has strong activities of these enzymes and uses glycogen at a high rate at the beginning of starvation; consequently, this bacterium does not survive very

long under starvation conditions. On the other hand, Arthrobacter globifor- mis stays viable for long periods of time under starvation conditions since this bacterium utilizes the reserve polysaccharide slowly with low activity of the debranching enzyme.

Some fungi synthesize trehalose as their reserve material. This disaccharide is a glucose dimer with an 2-1,1-linkage. This disacchar- ide is not a reserve material in prokaryotes, but many bacteria can use trehalose as their sole carbon and energy source. Some bacteria, including Desulfovibrio halophilus and several purple sulfur and non- sulfur bacteria, synthesize trehalose under high osmotic pressure as a compatible solute, but this disaccharide is not regarded as a reserve material.Trehalose-6-phosphate synthase condenses UDP-glucose and glu- cose-6-phosphate to trehalose-6-phosphate that is dephosphorylated to trehalose by trehalose-6-phosphatase. This disaccharide is metabo- lized in three different ways depending on the organism. Trehalase hydrolyzes it to two molecules of glucose, while trehalose phosphor- ylase cleaves it to glucose and glucose-1-phosphate. In other organ- isms, trehalose is phosphorylated to trehalose-6-phosphate by the action of trehalose kinase before being cleaved to glucose-6- phosphate and glucose by a hydrolase.

DISCUSSION

Lipid Reserve Materials

Poly-β-hydroxyalkanoates, triacylglycerides, wax esters and hydrocarbons are synthesized as reserve materials in bacteria. These lipophilic compounds are accumulated as inclusion bodies in the cytoplasm. Many proteins are associated with the inclusion bodies. These are mostly enzymes involved in the metabolism of the reserve material. PHAs are the most common reserve material in prokaryotes but are not found in eukaryotes. On the other hand, only a few prokaryotes have the property of accumulating triacylgly- cerides as a reserve material, a property that is widespread in eukar- yotes. Wax esters are accumulated mostly in bacteria [7]–[9].

Poly-β-hydroxyalkanoate

Generally, the PHA in the inclusion body is a macro- molecule with a molecular weight higher than 6 daltons. PHA is an ideal reserve material since this water-insoluble polymer does not increase the intracellular osmotic pressure and the energy content is higher than that of carbohydrates. Under nitrogen- and oxygen-limited conditions, some bacteria including Ralstonia eutropha and Azotobacter bei- jerinckii accumulate up to % of their dry cell weight as PHA. An industrial process has been developed to produce PHA as a biodegrad- able plastic using a strain of Ralstonia eutropha.

PHB is synthesized through the polymerization of 3-hydroxybutyrate, which is a condensation product of acetyl-CoA in a series of reactions similar to the clostridial butyrate fermentation.Azotobacter beijerinckii metabolizes carbohydrates though the ED pathway, and the resulting NADH is reoxidized through the electron transport system generating a proton motive force when the oxygen supply is not limited. When oxygen is limited, NADH is accumulated to inhibit enzymes reducing NADþ including glucose-6-phosphate dehydrogenase, citrate synthase and isocitrate dehydrogenase.When oxygen is limiting, acetyl-CoA is directed toward PHB synthesis reoxidizing NADH. For this reason, PHB is regarded as a reserve material and as an electron sink allowing the bacterium to continue glycolytic metabolism.PHB depolymerase removes 3-hydroxybutyrate from PHB, and the product is oxidized to acetoacetate. Coenzyme A transferase activates acetoacetate to acetoacetyl-CoA consuming succinyl-CoA. Since acet- oacetyl-CoA is involved both in

synthesis and degradation, the enzymes related to this intermediate need to be strongly regulated to avoid a futile cycle wasting a high energy bond in the form of succinyl-CoA

Triacylglyceride

TAG can serve as a reserve material in eukaryotes as well as in prokaryotes. Among prokaryotes, TAG is found mainly in the actino- mycetes, including species of Mycobacterium, Nocardia, Rhodococcus, Streptomyces, Micromonospora and Gordonia. Among Gram-negative bac- teria, species of Acinetobacter accumulate small amounts. A strain of Rhodococcus opacus accumulates TAG up to % of the cell dry weight. TAG is synthesized from glycerol-3-phosphate and acyl-ACP. Phosphatidic acid is produced as in phospholipid synthesis before phosphatidate phosphatase removes the phos- phate group. Finally the third acyl group is added to diacylglycerol from acyl-ACP by the action of diacylglycerol acyltransferase. DGAT is believed to be associated with the cytoplasmic membrane. TAG is accumulated with an excess of carbon source but when nutri-ents other than the carbon source are limited as with PHA.

Wax ester is synthesized by various bacteria including species of Acinetobacter, Moraxella, Micrococcus, Corynebacterium and Nocardia. Under nitrogen-limited conditions, a strain of Acinetobacter calcoaceti- cus accumulates up to % of the cell dry weight. It is not known how WE is synthesized, but wax ester synthase is associated with the cytoplasmic membrane like DGAT.Hydrocarbons are accumulated by various microorganisms, espe- cially algae. These microbial hydrocarbons are either isoprenoids or alkanes. A strain of Vibrio furnissii isolated from a sewage works accumulated lipid material extracellularly to 1.2 times its cell dry weight. Half of the extracellular lipid consisted of alkanes with a carbon number of –.

Polypeptides as Reserve Materials

Cyanobacteria utilize not only the usual reserve materials such as glycogen, poly-β-hydroxyalkanoate and polyphosphate, but also peptides. Many cyanobacteria accumulate cyanophycin as a reserve material for carbon and nitrogen. Cyanophycin has a struc- ture composed of polyaspartate, each monomer of which is linked with a molecule of arginine. This peptide has a molecu- lar weight of between ,0 and 5,0 daltons. This peptide is synthesized under phosphate- or sulfate-limited conditions with nitrogen and light in excess. When the synthesis of protein and nucleic acids is inhibited, cyanophycin production is activated. Its synthesis is not inhibited by tetracycline, showing that cyanophycin is synthesized through a non-ribosomal mechanism.

When the nitrogen supply is limited, cyanophycin is degraded producing ammonia and carbon dioxide. It is not known if energy is conserved during oxidation of the amino acids. In heterocystous cyanobacteria, the heterocysts have a higher enzyme activity for cyanophycin synthesis than the vegetative cells.Phycocyanin is a pigment peptide in the antenna molecule. When the nitrogen supply is limited, this peptide is also degraded as a nitrogen source. Cyanobacteria have a bluish-green colour under nor- mal conditions but become yellowish-green under nitrogen-limited conditions because the blue coloured phycocyanin is degraded. It should be noted that phycocyanin is not a ‘true’ reserve material according to the definition given earlier.

Polyphosphate

It has been known for a long time that many bacteria have cytoplasmic granules that are stainable with basic dyes such as toluidine blue. These granules are composed of polyphosphate and are known in prokaryotes and eukaryotes. The number of phosphate

residues ranges from two to over a million. Polyphosphate is consumed when the phosphate supply is limited, and in some organisms this can substitute for ATP in energy-requiring reactions. Polyphosphate has functions other than just as a phosphate and energy reserve material. These include regulation of the concentration of cytoplasmic cations because of its strong anionic properties, stabilization of the cytoplas- mic membrane and regulation of gene expression and enzyme activity. Polyphosphate is synthesized through the transfer of phosphate from ATP or from an intermediate of the glycolytic pathway, 1,3-diphosphoglycerate, to the existing polyphosphate.

Polyphosphate synthesis is under elaborate regulation. In Escherichia coli, polyphosphate is synthesized during the stringent response with an increase in ppGpp concentration. Polypho-sphate is not synthesized in a PhoB mutant. PhoB is the regulator protein of the two-component pho system. The free energy change in polyphosphate hydrolysis is kJ/mol phosphate and this is bigger than that of ATP and smaller than that of 1, 3-diphosphoglycerate. As mentioned above, polyphosphate hydrolysis is coupled to the syn. Many bacteria differentiate into resting cells when the growth envir- onment becomes unfavourable. These include spores in low G þ C Gram-positive bacteria and actinomycetes, cysts, and viable but non-culturable cells. In addition to the formation of resting cells, some bacteria differentiate into other specialized cells including bacter- oids in symbiotic nitrogen-fixing bacteria, heterocysts in cyanobac- teria, swarmer cells in Caulobacter cereus, and fruiting bodies in myxobacteria.

Sporulation in Bacillus Subtilis

Some low G þ C Gram-positive bacteria, including species of Bacillusand Clostridium, form spores under nutrient-limited and other adverse growth conditions. Spores can maintain their viability for many years.As in other cell differentiation processes, sporulation is the result of a complex regulated process which includes signal transduction from environmental and physiological factors. During the spore- forming process the cells cannot propagate. Sporulation is regulated through a series of phosphate transfers known as a phosphorelay. Phosphorylated Spo0 A plays a pivotal role in sporulation, activating the genes for sporulation and repressing the genes for vegetative growth. Spo0 A is phosphorylated through sporulation kinase A or B, Spo0 F and Spo0 B.

Cysts

In some bacteria such as Azotobacter and Cytophaga, resting cells known as cysts are formed. A cyst differs from a vegetative cell both in size and morphology. A cyst is resistant against desiccation and UV like a spore, but is not heat resistant. The outer wall of a cyst consists of protein and alginate. Genes for alginate production are transcribed by RNA polymerase with the extracytoplasmic function σ-factor, σE.

Viable but non-Culturable Cells

Many soil and marine bacteria are in a living state but fail to grow on routine bacteriological media. They are referred to as viable but non- cultural cells. Some authors claim that VBNC is not a proper term since some of these cells can be ‘resuscitated’ under proper conditions. Instead, the term ‘yet-to-be-cultured’ can be used. The VBNC state differs from the starvation survival state, which remains fully culturable even though the metabolic activity is very low, as in VBNC cells. Actively growing cells of various bacteria may enter the VBNC state under diverse environmental stresses including starvation, incubation outside the temperature range of growth, high osmotic pressure and exposure to white light. These stresses might be lethal if the cells did not enter this dormant state.In addition to the failure to

grow on routine bacteriological media, cells in the VBNC state differ from actively growing cells both morphologically and physiologically. VBNC cells are smaller and have increased cell wall cross-linking and extensively modified cytoplasmic membranes with altered fatty acid composition

KinA and KinB consume ATP to phosphorylate Spo0 F that transfers phosphate to Spo0 A through Spo0 B. This phosphate transfer is referred to as a phosphorelay. The phosphorylated Spo0 A represses the genes for vegetative cell growth and activates the genes for spore formation. The phosphorelay is under the control of four different steps. KapB regulates the activity of the membrane-bound KinB, and the cytoplasmic enzyme KinA is under the control of antikinase and anti-antikinase. The phosphorylated Spo0 A is dephosphorylated by Spo0 E and dephosphorylation of Spo0 F-P is under the control of RapA and RapB. It is not fully understood how the signal is transduced to KapB, KipI, KipR, Spo0 E, RapA and RapB.KapB, kinase-associated protein B; KinA, sporulation kinase A; KinB, sporulation kinase B; KipI, antikinase ; KipR, anti- antikinase ; RapA, response regulator aspartate phosphatase A; RapB, response regulator aspartate phosphatase B; PhrA, phosphatase inhibitor; Spo0 A, two-component response regulator; Spo0 B, sporulation initiation phosphotransferase; Spo0 E, negative sporulation regulatory phosphatase; Spo0 F, two-component response regulator; ComA, two-component response regulator.

Nanobacteria

In seawater and soil, cells with a size of less than 0.2 µm have been described for many years. These do not form colonies on routine solid media. They are referred to as ultramicrobacteria or nano- bacteria. It was believed that they are in a kind of VBNC statethat developed under nutrient-limited conditions. However, a sea- water isolate, Sphingomonas alaskensis, has cells of this size while it grows. This bacterium is an oligotroph with a very small genome. This bacterium loses viability under nutrient-rich condi- tions, and is resistant to stresses including heat, hydrogen peroxide and ethanol.

Programmed cell death in bacteria

Programmed cell death, or apoptosis, is a suicide process of active cells in multicellular eukaryotes. Cells no longer needed during a developmental process or those damaged by heat or other lethal agents are destroyed through proteolysis for the preservation of the tissue and/or the individual organism.Although PCD has been regarded as a eukaryotic phenomenon, similar processes are known in some bacteria. Bacteroids and hetero- cysts do not divide or return to vegetative cells. When their function is no longer required, they are subject to auto- lysis like the parental cells of endospore formers. These are examples of PCD in bacteria that are similar to eukaryotic developmental PCD. Damaged cells are eliminated through autolysis as in a PCD mechan- ism. Damaged cells that have survived toxic stress induced by various compounds could consume nutrients but would produce few if any offspring which would be a burden for the whole population. PCD of damaged cells therefore benefits the entire population. Bacteria thus appear to be able to exhibit social behaviour. Many live in large, complex and organized communities such as biofilms, while fruiting bodies arise through the initiation of developmental processes that are directly analogous to multicellular eukaryotic organisms. Multi- cellularity in the bacterial world, in its various forms, might be more the norm than a deviation. With some of these known similarities to the developmental processes of eukaryotes, ‘altruistic’ behaviour in bacteria might be a predictable rather than an unexpected phenomenon.

CONCLUSION

In Staphylococcus aureus, various genes have been identified that are related to the regulation of autolysis and murein hydrolase. The genes are believed to code for regulatory proteins involved in PCD. Actively metabolizing bacterial cells maintain a certain level of proton motive force with a relatively low pH microenvironment at the cell surface. When a cell is damaged with dissipation of the proton motive force, changes in the cell surface pH are sensed and transduced by a two-component system consisting of autolysin sensor kinase and sensory transduction protein. It has been hypothesized that this signal represses the activity of antiholin-like protein A and B, and activates holin- like protein A and B. LrgA and LrgB inhibit murein hydrolase activity while the enzyme activity is activated by CidA and CidB for PCD. The homologues of lrgAB have been identified in many prokaryotes, including archaea and Gram-positive and Gram-negative bacteria, indicating that PCD is probably widespread in prokaryotes.

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CHAPTER 11

EXPLORING THE PROCESS OF FERMENTATION

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ABSTRACT:

Fermentation is a metabolic process that converts sugar to acids, gases or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product like enzyme, vaccines, antibiotics, food product/additive etc. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology. Fermentation takes place in the lack of oxygen and becomes the cell's primary means of ATP production. It turns NADH and pyruvate produced in the glycolysis step into NAD+ and various small molecules depending on the type of fermentation. In the presence of O2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone.

KEYWORDS:

Fermentation, NADH, Microorganisms, Bacteria.

INTRODUCTION

For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen [1]–[3].Pyruvate is CH3COCOO Pi is inorganic phosphate. Two ADP molecules and two Pi are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD+ are also reduced to NADH. In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane via the electron transport chain. Glycolysis has substrate-level phosphorylation.Humans have used fermentation to produce food and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation can even occur within the stomachs of animals, such as humans.

Definitions of Fermentation

To many people, fermentation simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, one might say it was 'off' or fermented. Here are some definitions of fermentation. They range from informal, general usage to more scientific definitions.

1. Preservation methods for food via microorganisms.
2. Any process that produces alcoholic beverages or acidic dairy products.

3. Any large-scale microbial process occurring with or without air.
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions.
5. Any metabolic process that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor.

Examples of Fermentation

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption. The antibiotic activity of hops also inhibits aerobic metabolism in yeast. Fermentation react NADH with an endogenous, organic electron acceptor. Usually this is pyruvate formed from the sugar during the glycolysis step. During fermentation, pyruvate is metabolized to various compounds through several processes:

1. Ethanol fermentation, aka alcoholic fermentation, is the production of ethanol and carbon dioxide
2. Lactic acid fermentation refers to two means of producing lactic acid
3. Homolactic fermentation is the production of lactic acid exclusively
4. Heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide, and hydrogen gas. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

Ethanol Fermentation

The chemical equation below shows the alcoholic fermentation of glucose, whose chemical formula is C6HO6. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

Lactic Acid Fermentation

Homolactic fermentation is the simplest type of fermentation. The pyruvate from glycolysis undergoes a simple redox reaction, forming lactic acid. It is unique because it is one of the only respiration processes to not produce a gas as a byproduct. Overall, one molecule of glucose is converted to two molecules of lactic acid: C6HO6→2CH3CHOHCOOHIt occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some kinds of bacteria and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste. These lactic acid bacteria can carry out either homolactic fermentation, where the end-product is mostly lactic acid, or

1. The acidity of lactic acid impedes biological processes; this can be beneficial to the fermenting organism as it drives out competitors who are unadapted to the acidity; as a result the food will have a longer shelf-life; however, beyond a certain point, the acidity starts affecting the organism that produces it.

2. The high concentration of lactic acid drives the equilibrium backwards, decreasing the rate at which fermentation can occur, and slowing down growth
3. Ethanol, that lactic acid can be easily converted to, is volatile and will readily escape, allowing the reaction to proceed easily. CO2 is also produced, however it's only weakly acidic, and even more volatile than ethanol.
4. Acetic acid is acidic, and not as volatile as ethanol; however, in the presence of limited oxygen, its creation from lactic acid releases a lot of additional energy. It is a lighter molecule than lactic acid that forms fewer hydrogen bonds with its surroundings, and thus more volatile and will also allow the reaction to move forward more quickly.
5. If propionic acid, butyric acid and longer monocarboxylic acids are produced, the amount of acidity produced per glucose consumed will decrease, as with ethanol, allowing faster growth.

Aerobic Respiration

In aerobic respiration, the pyruvate produced by glycolysis is oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and is not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD+; lactic acid fermentation is one of these pathways.

Hydrogen Gas Production in Fermentation

Hydrogen gas is produced in many types of fermentation, as a way to regenerate NAD+ from NADH. Electrons are transferred to ferredoxin, which in turn is oxidized by hydrogenase, producing H2. Hydrogen gas is a substrate for methanogens and sulfate reducers, which keep the concentration of hydrogen low and favor the production of such an energy-rich compound, but hydrogen gas at a fairly high concentration can nevertheless be formed, as in flatus.This disproportionation reaction is catalysed by methanogen archaea in their fermentative metabolism. One electron is transferred from the carbonyl function of the carboxylic group to the methyl group of acetic acid to respectively produce CO2 and methane gas [4]–[6].

History of Fermentation

The use of fermentation, particularly for beverages, has existed since the Neolithic and has been documented dating from – BCE in Jiahu, China, BCE in Georgia, BCE in ancient Egypt, BCE in Babylon, BCE in pre-Hispanic Mexico, and BC in Sudan. Fermented foods have a religious significance in Judaism and Christianity. The Baltic god Rugutis was worshiped as the agent of fermentation.Louis Pasteur, during the s and s, showed that fermentation is initiated by living organisms in a series of investigations. In, Pasteur showed that lactic acid fermentation is caused by living organisms. In, he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization. In, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, "Etudes sur la Bière", which was translated into English in as "Studies on fermentation". He defined fermentation as "Life without air", but correctly showed that specific types of microorganisms because specific types of fermentations and specific end- products.

Although showing fermentation to be the result of the action of living microorganisms was a breakthrough, it did not explain the basic nature of the fermentation process, or prove that it is caused by the microorganisms that appear to be always present. Many scientists, including Pasteur, had unsuccessfully attempted to extract the fermentation enzyme from yeast. Success came in when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement that this "dead" liquid would ferment a sugar solution, forming carbon dioxide and alcohol much like living yeasts. Buechner's results are considered to mark the birth of biochemistry. The "unorganized ferments" behaved just like the organized ones. From that time on, the term enzyme came to be applied to all ferments. It was then understood that fermentation is caused by enzymes that are produced by microorganisms. In, Buechner won the Nobel Prize in chemistry for his work.

Advances in microbiology and fermentation technology have continued steadily up until the present. For example, in the late s, it was discovered that microorganisms could be mutated with physical and chemical treatments to be higher-yielding, faster-growing, tolerant of less oxygen, and able to use a more concentrated medium. Strain selection and hybridization developed as well, affecting most modern food fermentations. Other approach to advancing the fermentation industry has been done by companies such as BioTork, a biotechnology company that naturally evolves microorganisms to improve fermentation processes. This approach differs from the more popular genetic modification, which has become the current industry standard.

Industrial Fermentation

Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation. The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for aerobic fermentation oxygen. Product recovery frequently involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. In some cases, production of biomass itself is the objective, as in the case of baker's yeast and lactic acid bacteria starter cultures for cheese making. In general, fermentations can be divided into four types:

1. Production of biomass
2. Production of extracellular metabolites
3. Production of intracellular components
4. Transformation of substrate

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

General Overview of Industrial Fermentation

In most industrial fermentations, the organisms are submerged in a liquid medium; in others, such as the fermentation of cocoa beans, coffee cherries, and miso, fermentation takes place on the moist surface of the medium. There are also industrial considerations related to the fermentation process. For instance, to avoid biological process contamination, the

fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate.

DISCUSSION

Phases of Microbial Growth

When a particular organism is introduced into a selected growth medium, the medium is inoculated with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the lag phase. Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase. After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or continuously increasing concentrations of toxic substances. This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state. The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells. Unless other micro- organisms contaminate the culture, the chemical constitution remains unchanged. If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die off. The total amount of biomass may not decrease, but the number of viable organisms will decrease.

Fermentation Medium

The microbes used for fermentation grow in specially designed growth medium which supplies the nutrients required by the organisms. A variety of media exists, but invariably contains a carbon source, a nitrogen source, water, salts, and micronutrients. In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components. Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts. Cost is also an important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids. The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.

Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require. Yeast extract is a common source of micronutrients and vitamins for fermentation media. Inorganic nutrients, including trace

elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are used. Fermentations which produce large amounts of gas will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches. To prevent this foam from occurring or accumulating, antifoaming agents may be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum. When metal ions are present in high concentrations, use of a chelating agent may be necessary.

Production of Biomass

Microbial cells or biomass is sometimes the intended product of fermentation. Examples include single cell protein, baker's yeast, Lactobacillus, E. coli, and others. In the case of single-cell protein, algae are grown in large open ponds which allow photosynthesis to occur. If the biomass is to be used for inoculation of other fermentations, care must be taken to prevent mutations from occurring.

Production of extracellular metabolites

Microbial metabolites can be divided into two groups: those produced during the growth phase of the organism, called primary metabolites and those produced during the stationary phase, called secondary metabolites. Some examples of primary metabolites are ethanol, citric acid, glutamic acid, lysine, vitamins and polysaccharides. Some examples of secondary metabolites are penicillin, cyclosporin A, gibberellin, and lovastatin.

Primary Metabolites

Primary metabolites are compounds made during the ordinary metabolism of the organism during the growth phase. A common example is ethanol or lactic acid, produced during glycolysis. Citric acid is produced by some strains of Aspergillus niger as part of the citric acid cycle to acidify their environment and prevent competitors from taking over. Glutamate is produced by some Micrococcus species, and some Corynebacterium species produce lysine, threonine, tryptophan and other amino acids. All of these compounds are produced during the normal "business" of the cell and released into the environment. There is therefore no need to rupture the cells for product recovery.

Secondary Metabolites

Secondary metabolites are compounds made in the stationary phase; penicillin, for instance, prevents the growth of bacteria which could compete with Penicillium molds for resources. Some bacteria, such as Lactobacillus species, are able to produce bacteriocins which prevent the growth of bacterial competitors as well. These compounds are of obvious value to humans wishing to prevent the growth of bacteria, either as antibiotics or as antiseptics. Fungicides, such as griseofulvin are also produced as secondary metabolites. Typically secondary metabolites are not produced in the presence of glucose or other carbon sources which would encourage growth, and like primary metabolites are released into the surrounding medium without rupture of the cell membrane.

Production of Intracellular Components

Of primary interest among the intracellular components are microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase and many others. Recombinant proteins, such as insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase and others are also made this

way. The largest difference between this process and the others is that the cells must be ruptured at the end of fermentation, and the environment must be manipulated to maximize the amount of the product. Furthermore, the product must be separated from all of the other cellular proteins in the lysate to be purified.

Transformation of Substrate

Substrate transformation involves the transformation of a specific compound into another, such as in the case of phenylacetylcarbinol, and steroid biotransformation, or the transformation of a raw material into a finished product, in the case of food fermentations and sewage treatment.

Food Fermentation

Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., can be dated to more than seven thousand years ago. They were developed long before man had any knowledge of the existence of the microorganisms involved. Some foods such as Marmite are the byproduct of the fermentation process, in this case in the production of beer.

Ethanol Fuel

Fermentation is the main source of ethanol in the production of Ethanol fuel. Common crops such as sugar cane, potato, cassava and corn are fermented by yeast to produce ethanol which is further processed to become fuel.

Sewage Treatment

In the process of sewage treatment, sewage is digested by enzymes secreted by bacteria. Solid organic matters are broken down into harmless, soluble substances and carbon dioxide. Liquids that result are disinfected to remove pathogens before being discharged into rivers or the sea or can be used as liquid fertilizers. Digested solids, known also as sludge, is dried and used as fertilizer. Gaseous byproducts such as methane can be utilized as biogas to fuel electrical generators. One advantage of bacterial digestion is that it reduces the bulk and odor of sewage, thus reducing space needed for dumping. The main disadvantage of bacterial digestion in sewage disposal is that it is a very slow process.

Agricultural Feed

A wide variety of agroindustrial waste products can be fermented to use as food for animals, especially ruminants. Fungi have been employed to break down cellulosic wastes to increase protein content and improve in vitro digestibility [7]–[9].

CONCLUSION

Fermentation takes place in the lack of oxygen and becomes the cell's primary means of ATP production. It turns NADH and pyruvate produced in the glycolysis step into NAD+ and various small molecules depending on the type of fermentation. In the presence of O2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen. Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations the carbon source may be an alcohol or something else altogether. For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor, sugar cane juice, or sugar beet juice are used to minimize costs. More

sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product. Organisms meant to produce enzymes such as beta galactosidase, invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity.

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CHAPTER 12

EXPLORING THE ROLE AND APPLICATIONS OF BIOASSAY

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ABSTRACT:

Bioassay, or biological standardization is a type of scientific experiment. A bioassay involves the use of live animal or plant or tissue or cell to determine the biological activity of a substance, such as a hormone or drug. Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs and in monitoring environmental pollutants. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. A bioassay can also be used to determine the concentration of a particular constitution of a mixture that may cause harmful effects on organisms or the environment.Bioassays are procedures that can determine the concentration or purity or biological activity of a substance such as vitamin, hormone or plant growth factor by measuring the effect on an organism, tissue, cells, enzyme or receptor. Bioassays may be qualitative or quantitative. Qualitative bioassays are used for assessing the physical effects of a substance that may not be quantified, such as seeds fail to germinate or develop abnormally deformity.

KEYWORDS:

Applications, Growth Factor, Bioassay, Biological Standardization.

INTRODUCTION

An example of a qualitative bioassay includes Arnold Adolph Berthold's famous experiment on castrated chickens. This analysis found that by removing the testicles of a chicken, it would not develop into a rooster because the endocrine signals necessary for this process were not available. Quantitative bioassays involve estimation of the dose-response curve, how the response changes with increasing dose. That dose-response relation allows estimation of the dose or concentration of a substance associated with a specific biological response, such as the LC. Quantitative bioassays are typically analyzed using the methods of biostatistics[1], [2].

Purpose of Bioassays

1. Measurement of the pharmacological activity of new or chemically undefined substances
2. Investigation of the function of endogenous mediators
3. Determination of the side-effect profile, including the degree of drug toxicity
4. Measurement of the concentration of known substances
5. Assessing the amount of pollutants being released by a particular source, such as wastewater or urban runoff.
6. Determining the specificity of certain enzymes to certain substrates.

Types of Bioassays

Bioassays are of two types:Quantal: A quantal assay involves an "all or none response".

Graded: Graded assays are based on the observation that there is a proportionate increase in the observed response following an increase in the concentration or dose. The parameters employed in such bioassays are based on the nature of the effect the substance is expected to produce. For example: contraction of smooth muscle preparation for assaying histamine or the study of blood pressure response in case of adrenaline. A graded bioassay can be performed by employing any of the below-mentioned techniques[3], [4]. The choice of procedure depends on:

1. The precision of the assay required
2. The quantity of the sample substance available
3. The availability of the experimental animals.

Bioassay Techniques

1. Matching Bioassay
2. Interpolation Method
3. Bracketing Method
4. Multiple Point Bioassay
5. Divided bioassay

Matching Bioassay

It is the simplest type of the bioassay. In this type of bioassay, response of the test substance taken first and the observed response is tried to match with the standard response. Several responses of the standard drug are recorded till a close matching point to that of the test substance is observed. A corresponding concentration is thus calculated. This assay is applied when the sample size is too small. Since the assay does not involve the recording of concentration response curve, the sensitivity of the preparation is not taken into consideration. Therefore, precision and reliability is not very good[5], [6].

Interpolation Bioassay

Bioassays are conducted by determining the amount of preparation of unknown potency required to produce a definite effect on suitable test animals or organs or tissue under standard conditions. This effect is compared with that of a standard. Thus the amount of the test substance required to produce the same biological effect as a given quantity the unit of a standard preparation is compared and the potency of the unknown is expressed as a % of that of the standard by employing a simple formula[7], [8].Many times, a reliable result cannot be obtained using this calculation. Therefore, it may be necessary to adopt more precise methods of calculating potency based upon observations of relative, but not necessarily equal effects, likewise, statistical methods may also be employed. The data on which bioassay are based may be classified as quantal or graded response. Both these depend ultimately on plotting or making assumption concerning the form of DRC.

Environmental Bioassays

Environmental bioassays are generally a broad-range survey of toxicity. A toxicity identification evaluation is conducted to determine what the relevant toxicants are. Although bioassays are beneficial in determining the biological activity within an organism, they can often be time- consuming and laborious. Organism-specific factors may result in data that are not applicable to others in that species. For these reasons, other biological techniques are often employed, including radio-immunoassays.

Quality Control

Quality control, or QC for short, is a process by which entities review the quality of all factors involved in production. ISO defines quality control as "A part of quality management focused on fulfilling quality requirements".This approach places an emphasis on three aspects:

1. Elements such as controls, job management, defined and well managed processes, performance and integrity criteria, and identification of records.
2. Competence, such as knowledge, skills, experience, and qualifications.
3. Soft elements, such as personnel, integrity, confidence,organizational culture, motivation, team spirit, and quality relationships.

Controls include product inspection, where every product is examined visually, and often using a stereo microscope for fine detail before the product is sold into the external market. Inspectors will be provided with lists and descriptions of unacceptable product defects such as cracks or surface blemishes for example. The quality of the outputs is at risk if any of these three aspects is deficient in any way.Quality control emphasizes testing of products to uncover defects and reporting to management who make the decision to allow or deny product release, whereas quality assurance attempts to improve and stabilize production to avoid, or at least minimize, issues which led to the defect in the first place. For contract work, particularly work awarded by government agencies, quality control issues are among the top reasons for not renewing a contract.

Shelf Life of Products

Shelf life is the length of time that a commodity may be stored without becoming unfit for use, consumption, or sale. In other words, it might refer to whether a commodity should no longer be on a pantry shelf, or just no longer on a supermarket shelf. It applies to cosmetics, foods and beverages, medical devices, medicines, explosives, pharmaceutical drugs, chemicals, car tires, batteries, and many other perishable items. In some regions, an advisory best before, mandatory use by, or freshness date is required on packaged perishable foods.

Background

Shelf life is the recommended maximum time for which products or fresh produce can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected conditions of distribution, storage and display. Most expiration dates are used as guidelines based on normal and expected handling and exposure to temperature. Use prior to the expiration date does not guarantee the safety of a food or drug, and a product is not necessarily dangerous or ineffective after the expiration date.According to the USDA, "canned foods are safe indefinitely as long as they are not exposed to freezing temperatures, or temperatures above °F. If the cans look ok, they are safe to use. Discard cans that are dented, rusted, or swollen. High-acid canned foods will keep their best quality for to months; low-acid canned foods for 2 to 5 years °F.

"Sell by date" is a less ambiguous term for what is often referred to as an "expiration date". Most food is still edible after the expiration date. A product that has passed its shelf life might still be safe, but quality is no longer guaranteed. In most food stores, waste is minimized by using stock rotation, which involves moving products with the earliest sell by date from the warehouse to the sales area, and then to the front of the shelf, so that most shoppers will pick them up first and thus they are likely to be sold before the end of their shelf life. This is

important, as consumers enjoy fresher goods, and furthermore some stores can be fined for selling out of date products; most if not all would have to mark such products down as wasted, resulting in a financial loss.Shelf life depends on the degradation mechanism of the specific product. Most can be influenced by several factors: exposure to light, heat, moisture, transmission of gases, mechanical stress and contamination by things such as micro-organisms. Product quality is often mathematically modelled around a parameter.

For some foods, health issues are important in determining shelf life. Bacterial contaminants are ubiquitous, and foods left unused too long will often be contaminated by substantial amounts of bacterial colonies and become dangerous to eat, leading to food poisoning. However, shelf life alone is not an accurate indicator of how long the food can safely be stored. For example, pasteurized milk can remain fresh for five days after its sell-by date if it is refrigerated properly. In contrast, if milk already has harmful bacteria, the use-by dates become irrelevant.The expiration date of pharmaceuticals specifies the date the manufacturer guarantees the full potency and safety of a drug. Most medications continue to be effective and safe for a time after the expiration date. A rare exception is a case of renal tubular acidosis purportedly caused by expired tetracycline. A study conducted by the U.S. Food and Drug Administration covered over 0 drugs, prescription and over-the-counter. The study showed that about % of them were safe and effective as long as years past their expiration dates. Joel Davis, a former FDA expiration-date compliance chief, said that with a handful of exceptions - notably nitroglycerin, insulin and some liquid antibiotics - most expired drugs are probably effective.

DISCUSSION

Shelf life is not significantly studied during drug development, and drug manufacturers have economic and liability incentives to specify shorter shelf lives so that consumers are encouraged to discard and repurchase products. One major exception is the Shelf Life Extension Program of the U.S. Department of Defense which commissioned a major study of drug efficacy from the FDA starting in the mid-s. One criticism is that the U.S. Food and Drug Administration refused to issue guidelines based on SLEP research for normal marketing of pharmaceuticals even though the FDA performed the study. The SLEP and FDA signed a memorandum that scientific data could not be shared with the public, public health departments, other government agencies, and drug manufacturers. State and local programs are not permitted to participate. The failure to share data has caused foreign governments to refuse donations ofexpired medications. One exception occurred during the Swine Flu Epidemic when the FDA authorized expired Tamiflu based on SLEP Data. The SLEP discovered that drugs such as Cipro remained effective nine years after their shelf life, and, as a cost-saving measure, the US military routinely uses a wide range of SLEP tested products past their official shelf life if drugs have been stored properly.Preservatives and antioxidants may be incorporated into some food and drug products to extend their shelf life. Some companies use induction sealing and vacuum/oxygen-barrier pouches to assist in the extension of the shelf life of their products where oxygen causes the loss.The DoD Shelf-Life Program defines shelf-life as.

The total period of time beginning with the date of manufacture, date of cure , date of assembly, or date of pack , and terminated by the date by which an item must be used or subjected to inspection, test, restoration, or disposal action; or after inspection/laboratory test/restorative action that an item may remain in the combined wholesale and retail storage systems and still be suitable for issue or use by the end user. Shelf-life is not to be confused with service-life. Shelf life is often specified in conjunction with a specific product, package,

and distribution system. For example, an MRE field ration is designed to have a shelf life of three years at °F and six months at 0 °F .

Temperature Control

Nearly all chemical reactions can occur at normal temperatures. However most reactions are accelerated by high temperatures and the degradation of foods and pharmaceuticals is no exception. The same applies to the breakdown of many chemical explosives into more unstable compounds. Nitroglycerine is notorious. Oldexplosives are thus more dangerous than more recently manufactured explosives. Rubber products also degrade as sulphur bonds induced during vulcanization revert; this is why old rubber bands and other rubber products soften and get crispy, and lose their elasticity as they age.

The usually quoted rule of thumb is that chemical reactions double their rate for each temperature increase of °C because activation energy barriers are more easily surmounted at higher temperatures. However, as with many rules of thumb, there are many caveats and exceptions. The rule works best for reactions with activation energy values around kJ/mole; many of these are important at the usual temperatures we encounter. It is often applied in shelf life estimation, sometimes wrongly. There is a widespread impression, for instance in industry, that "triple time" can be simulated in practice by increasing the temperature by °C , e.g., storing a product for one month at °C simulates three months at °C . This is mathematically incorrect), and in any case the rule is only a rough approximation and cannot always be relied on.The same is true, up to a point, of the chemical reactions of living things. They are usually catalyzed by enzymes which change reaction rates, but with no variation in catalytic action, the rule of thumb is still mostly applicable. In the case of bacteria and fungi, the reactions needed to feed and reproduce speed up at higher temperatures, up to the point that the proteins and other compounds in their cells themselves begin to break down, or denature, so quickly that they cannot be replaced. This is why high temperatures kill bacteria and other micro-organisms: 'tissue' breakdown reactions reach such rates that they cannot be compensated for and the cell dies. On the other hand, 'elevated' temperatures short of these result in increased growth and reproduction; if the organism is harmful, perhaps to dangerous levels.

Just as temperature increases speed up reactions, temperature decreases reduce them. Therefore, to make explosives stable for longer periods, or to keep rubber bands springy, or to force bacteria to slow down their growth, they can be cooled. That is why shelf life is generally extended by temperature control: and why some medicines and foods must be refrigerated. Since such storing of such goods istemporal in nature and shelf life is dependent on the temperature controlled environment, they are also referred to as cargo even when in special storage to emphasize the inherent time- temperature sensitivity matrix.Temperature data loggers and time temperature indicators can record the temperature history of a shipment to help estimate their remaining shelf life.According to the USDA, "foods kept frozen continuously are safe indefinitely."

Product Packaging

Barrier packaging can often help control or extend shelf life. When moisture content is a mechanism for product degradation, packaging with a low moisture vapor transmission rate and the use of desiccants help keep the moisture in the package within acceptable limits. When oxidation is the primary concern, packaging with a low oxygen transmission rate and the use of oxygen absorbers can help extend the shelf life. Produce and other products with respiration often require packaging with controlled barrier properties. The use of a modified

atmosphere in the package can extend the shelf life for some products. Some active packaging is also available with antibacterial properties.

Parallel Names

Best before or best by dates appear on a wide range of frozen, dried, tinned and other foods. These dates are only advisory and refer to the quality of the product, in contrast with use by dates, which indicate that the product may no longer be safe to consume after the specified date. Food kept after the best before date will not necessarily be harmful, but may begin to lose its optimum flavour and texture. Eggs are a special case, since they may contain salmonella which multiplies over time; they should therefore be eaten before the best before date, which is, in the USA, a maximum of days after the eggs are packed.

Sometimes the packaging process involves using pre-printed labels, making it impractical to write the best before date in a clearly visible location. In this case, wording like best before see bottom or best before see lid might be printed on the label and the date marked in a different location as indicated. Generally, foods that have a use by date written on the packaging must notbe eaten after the specified date. This is because such foods usually go bad quickly and may be injurious to health if spoiled. It is also important to follow storage instructions carefully for these foods. Bathroom products and toiletries usually state a time in months from the date the product is opened, by which they should be used. This is often indicated by a graphic of an open tub, with the number of months written inside. Similarly, some food products say "eat within X days of opening".

Open Dating

Open dating is the use of a date stamped on the package of a food product to help determine how long to display the product for sale. This benefits the consumer by ensuring that the product is of best quality when sold. An open date does not supersede a use-by date, if shown, which should still be followed.These dates are intended to help keep track of the stock in stores. Food that has passed its sell by or display until date, but has not yet reached its use by / best before date will still be edible, assuming it has been stored correctly. It is common practice in large stores to throw away such food, as it makes the stock control process easier; another common practice is for wholesalers to repurchase the expired product and resell it to discount stores at much lower clearance sale prices. These practices reduce the risk of customers unknowingly buying food without looking at the date, only to find out the next day that they cannot use it. Tampering with the posted date is illegal in many countries.Most stores will rotate stock by moving the products with the earliest dates to the front of shelving units, which encourages customers to buy them first and hopefully saves them from having to be either marked down or thrown away, both of which would result in financial loss.

Freshness Date

A freshness date is the date used in the American brewing industry to indicate either the date the beer was bottled or the date before which the beer should be consumed. Beer is perishable. It can be affected by light, air, or the action of bacteria. Although beer is not legally mandated in the United States to have a shelf life, freshness dates serve much the same purpose and are used as a marketing tool.

Beginnings of Freshness Dating

General Brewing Company of San Francisco marketed their Lucky Lager Beer as "Age Dated" as early as late . They stamped a date on each can lid to indicate that the beer was

brewed before that date. This was not to ensure that the beer was "fresh" but to ensure that it had been aged properly. So many breweries had rushed beer to market before it was ready when Prohibition ended, that customers were wary of getting "green" beer. The Boston Beer Company, maker of Samuel Adams, was among the first contemporary brewers to start adding freshness dates to their product line in . For ten years there was a slow growth in brewers adding freshness dates to their beer. The practice rapidly grew in popularity after the Anheuser-Busch company's heavily marketed "Born-On dates" starting in . Many other brewers have started adding freshness dates to their products, but there is no standard for what the date means. For some companies, the date on the bottle or can will be the date that the beer was bottled; others have the date by which the beer should be consumed.

Food Spoilage

This apple has decomposed to the point that it is not of a quality appealing to humans to eat. Spoilage is the process in which food deteriorates to the point in which it is not edible to humans or its quality of edibility becomes reduced. Various external forces are responsible for the spoilage of food. Food that is capable of spoiling is referred to as perishable food.

Reasons for Spoilage

Harvested foods decompose from the moment they are harvested due to attacks from enzymes, oxidation and microorganisms. These include bacteria, mold, yeast, moisture, temperature and chemical reaction.

Bacteria

Bacteria can be responsible for the spoilage of food. When bacteria break down the food, acids and other waste products are created in the process. While the bacteria itself may or may not be harmful, the waste products may be unpleasant to taste or may even be harmful to one's health.

Yeasts

Yeasts can be responsible for the decomposition of food with high sugar content. The same effect is useful in the production of various types of food and beverages, such as bread, yogurt, cider, and alcoholic beverages.

Signs of Spoilage

Signs of food spoilage may include an appearance different from the food in its fresh form, such as a change in color, a change in texture, an unpleasant odor, or an undesirable taste. The item may become softer than normal. If mold occurs, it is often visible externally on the item.

Consequences of Spoilage

Food poisoning and more properly known as "foodborne illness".

Prevention of spoilage

A number of methods of prevention can be used that can totally prevent, delay, or otherwise reduce food spoilage.

a. Food rotation system uses the first in first out method , which ensures that the first item purchased is the first item consumed.

b. Preservatives can expand the shelf life of food and can lengthen the time long enough for it to be harvested, processed, sold, and kept in the consumer's home for a reasonable length of time.
c. Refrigeration can increase the shelf life of certain foods and beverages, though with most items, it does not indefinitely expand it. Freezing can preserve food even longer, though even freezing has limitations.
d. A high-quality vacuum flask will keep coffee, soup, and other boiling-hot foods above the danger zone for over hours.
e. Canning of food can preserve food for a particularly long period of time, whether canned at home or commercially. Canned food is vacuum packed in order to keep oxygen out of the can that is needed to allow bacteria to break it down. Canning does have limitations, and does not preserve the food indefinitely.
f. Lactic acid fermentation also preserves food and prevents spoilage.

CONCLUSION

A bioassay is an analytical technique that assesses a substance's concentration or potency through its impact on living organisms such as plants, animals, or human cells and tissues (in vivo or in vitro). A bioassay might be direct or indirect, quantal or quantitative. The assay is quantal if the measured response is binary; otherwise, it is quantitative. Bioassays are employed to identify biological risks or to rate the quality of a mixture. They are frequently used to keep an eye on the environment's effects from wastewater discharges and water quality. The safety and environmental impact of new technologies and facilities are also evaluated using bioassays. The development of bioassays, which are used to assess potency, stability, and comparability between various methods, is a crucial component of creating biopharmaceutical pharmaceuticals.

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CHAPTER 13

REGULATION OF PROKARYOTIC GENE EXPRESSION

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ABSTRACT:

The cardinal rule of existence for any organism is economy. A cell need not waste energy by simultaneously synthesizing different carbohydrate utilization systems if only one carbohydrate is available. Likewise, it is wasteful to produce all the enzymes required to synthesize an amino acid if that amino acid is already available in the growth medium. Extravagant practices such as these will jeopardize survival of a bacterial species by making it less competitive with the more efficient members of its microbial microcosm. However, there are many instances when economy must be ignored. Microorganisms in nature more often than not find themselves in suboptimal environments or under chemical or physical attack. Consequently, in any given ecological niche, the successful microbe not only needs regulatory systems designed to maximize the efficiency of gene expression during times of affluence but must also sense danger and suspend those safeguards in favor of emergency systems that will remove the threat or minimize the ensuing damage. Economy is important, but backup plans ensure survival of the species.

KEYWORDS:

Bacteria, Bacterial Growth,Prokaryotic Gene Expression, Microbial Physiology.

INTRODUCTION

Prokaryotic gene expression is classically viewed as being controlled at two basic levels. DNA transcription and RNA translation. However, it will become apparent that mRNA degradation, modification of protein activity, and protein degradation also play important regulatory roles. This chapter deals with the basics of gene expression, describes some of the well-characterized regulatory systems, and illustrates how these control systems integrate and impact cell physiology[1]–[3].The most obvious place to regulate transcription is at or around the promoter region of a gene. By controlling the ability of RNA polymerase to bind to the promoter, or, once bound, to transcribe through to the structural gene, the cell can modulate the amount of message being produced and hence the amount of gene product eventually synthesized.

Transcriptional Control

The sequences adjacent to the actual coding region involved in this control are called regulatory regions. These regions are composed of the promoter, where transcription initiates, and an operator region, where a diffusible regulatory protein binds. Regulatory proteins may either prevent transcription or increase transcription. The regulatory proteins may also require bound effector molecules such as sugars or amino acids for activity. Repressor proteins produce negative control while activator proteins are associated with positive control.

Transcription initiation requires three steps: RNA polymerase binding, isomerization of a few nucleotides, and escape of RNA polymerase from the promoter region, allowing elongation of the message. Negative regulators usually block binding while activators interact with RNA polymerase, making one or more steps, often transition from closed to open complexes, more likely to occur [4]–[6].

An operon is several distinct genes situated in tandem, all controlled by a common regulatory region. The message produced from an operon is polycistronic in that the information for all of the structural genes will reside on one mRNA molecule. Regulation of these genes is coordinate, since their transcription depends on a common regulatory region that is, transcriptions of all components of the operon either increase or decrease together. Often genes that are components in a specific biochemical pathway do not reside in an operon but are scattered around the bacterial chromosome. Nevertheless, they may be controlled coordinately by virtue of the fact that they all respond to a common regulatory protein. Systems involving coordinately regulated, yet scattered, genes are referred to as regulons. It presents a schematic representation of negative-control versus positive- control regulatory circuits. Whether an operon is under negative or positive control, it can be referred to as inducible if the presence of some secondary effector molecule is required to achieve an increased expression of the structural genes. Likewise, an operon is repressible if an effector molecule must bind to the regulatory protein before it will inhibit transcription of the structural genes.

DNA-Binding Proteins

It is evident that regulation at the transcriptional level relies heavily on DNA-binding proteins. Studies conducted on many different regulatory proteins have revealed groups based on common structural features.

The Lac Operon: A Paradigm of Gene Expression

The operon responsible for the utilization of lactose as a carbon source, the lac operon, has been studied extensively and is of classical importance. Its examination led Jacob and Monod to develop the basic operon model of gene expression nearly half a century ago. Even now, understanding the many facets of its control underscores the economics of gene expression and reveals that no single operon acts in a metaphorical vacuum. It will become apparent that aspects of cell physiology beyond the simple notion of lactose availability govern the expression of this operon.

The product of the lacZ gene, ß-galactosidase, cleaves the ß-1,4 linkage of lactose, releasing the free monosaccharides. The enzyme is a tetramer of four identical subunits, each with a molecular weight of 6,0. Entrance of lactose into the cell requires the lac permease, the product of the lacY gene. The permease is hydrophobic and probably functions as a dimer. Mutations in either the lacZ or lacY genes are phenotypically Lac that is, the mutants cannot grow on lactose as a sole carbon source. The lacA locus is the structural gene for thiogalactoside transacetylase for which no definitive role has been assigned. The promoter and operator for the lac operon are lacP and lacO, respectively. The lacI gene codes for the repressor protein, and in this system is situated next to the lac operon. Often, regulatory loci encoding for diffusible regulator proteins map some distance from the operons they regulate. The lacI gene product functions as a tetramer.

The lac operator is bp in length and is adjacent to the ß-galactosidase structural gene. The complete sequence of the lacP-O region, including the C terminus of lacI and the N terminus of lacZ. The operator overlaps the promotor in that the lac repressor, when bound to the lac

operator in vitro, will protect part of the promoter region from nuclease digestion. The mechanism of repression remains somewhat controversial. There is evidence that under some in vitro conditions, RNA polymerase can bind to the promotor in the presence of lac repressor. Thus, one proposal is that the binding of repressor to the operator region situated between the promoter and lacZ physically blocks transcription by preventing the release of RNA polymerase from the promoter and its movement into the structural gene. Other evidence indicates that RNA polymerase and lac repressor cannot bind simultaneously, leading to a model whereby lac repressor simply competes with RNA polymerase for binding in the promoter/operator region.

DISCUSSION

Whatever the mechanism, induction of the lac operon, outlined, occurs when cells are placed in the presence of lactose. The low level of ß-galactosidase that is constitutively present in the cell will convert lactose to all lactose. Allolactose is the actual inducer molecule. The lac repressor is an allosteric molecule with distinct binding sites for DNA and the inducer. The binding of the inducer to the tetrameric repressor occurs whether the repressor is free in the cytoplasm or bound to DNA. Binding of the inducer to the repressor, however, allosterically alters the repressor, lowering its affinity for lacO DNA. Once the repressor is removed from lacO, transcription of lacZYA can proceed. Thus, the lac operon is a negatively controlled inducible system. It should be noted that experiments designed to examine induction of the lac operon usually take advantage of a gratuitous inducer such as isopropyl-ß-thiogalactoside — a molecule that will bind the repressor and inactivate it but is not itself a substrate for ß-galactosidase. This eliminates any secondary effects that the catabolism of lactose may have on lac expression.

There are several regulatory mutations that have been identified in the lac operon that serve to illustrate general concepts of gene expression. Mutations in the lacI locus can give rise to three observable phenotypes. The first and most obvious class of mutations results in an absence of, or a nonfunctional, repressor. This will lead to a constant synthesis of lacZYA message regardless of whether the inducer is present. This is referred to as constitutive expression of the lac operon. If the lac repressor prevents expression of the lac operon, then how does the cell allow for production of the small amount of β-galactosidase needed to make allolactose inducer and enough LacP permease to enable full induction when the opportunity arises?

lac promoter called P2. This promoter binds RNA polymerase tightly but initiates transcription poorly. Once initiation occurs from P2, RNA polymerase can transcribe past the LacI-bound operator and into the structural lacZYA genes. Note also that the message produced from P2 will contain a large palindromic region corresponding to the operator-binding site. The stem-loop structure that forms will sequester the lacZ. SD sequence, allowing only minimal production of β-galactosidase. This palindromic sequence is not produced from P1.

Catabolite Control: Sensing Energy Status

The lac operon has an additional, positive regulatory control system. The purpose of this control circuit is to avoid wasting energy by synthesizing lactose-utilizing proteins when there is an ample supply of glucose available. Glucose is the most efficient carbon source for E. coli. Since the enzymes for glucose utilization in E. coli are constitutively synthesized, it would be pointless for the cell to also make the enzymes for lactose catabolism when glucose and lactose are both present in the culture medium. The phenomenon can be visualized. This classic experiment shows. Initially growing on succinate with IPTG as an inducer of β-

galactosidase activity. The early part of the graph shows an increase in β-galactosidase activity due to the induction. Then, at the point indicated, glucose is added to one culture. A dramatic cessation of further β-galactosidase synthesis is observed followed by a partial resumption of synthesis. It was presumed that a catabolite of glucose was causing this phenomenon, hence the term catabolite repression. However, adding cyclic AMP simultaneously with glucose reduces catabolite repression.The phenomenon of catabolite repression is partly based on the fact that whenE. coli is grown on glucose, intracellular cAMP levels decrease, but when grown on an alternate carbon source such as succinate, cAMP levels increase.

Class I and Class II CRP-Dependent Genes

CRP, acting as a dimer, regulates more than 0 genes. The cAMP– CRP complex binds to target bp sequences located near or within CRP-dependent promoters. At these promoters, the cAMP– CRP complex activates transcription by making direct protein– protein interactions with RNA polymerase. Simple CRP-dependent promoters contain a single CRP-binding site and are grouped into two classes. Class I CRP-Dependent promoters have the CRP-binding site located upstream of the RNAP-binding site. Class II CRP-dependent promoters overlap the RNAP-binding site. At class I promoters, CRP activates transcription by contacting RNAP via a surface-exposed β turn on the downstream CRP subunit. The contact patch on CRP, called activating region 1, makes

Contact with the C-terminal domain to the RNAP α subunit. This serves to recruit RNAP to the promoter.The process of transcription activation at class II promoters is more complex. RNAP binds to promoter regions both upstream and downstream of the DNA-bound CRP, making multiple interactions with CRP. In this situation, AR1 in the upstream CRP subunit interacts with α-CTD situated upstream of CRP. The second activating region is a positively charged surface on the downstream CRP subunit. AR2 interacts with the N-terminal domain of the subunit but only at class II promoters.

Catabolite Control: The Gram-Positive Paradigm

Bacillus subtilis and other gram-positive microorganisms utilize a different system for catabolite repression. A protein called CcpA is able to differentially bind the phosphorylated forms of HPr. As part of the PTS transport system, HPr is phosphorylated at residue His- in the absence of carbohydrate transport. When a suitable carbohydrate is transported through the PTS system, HPr donates the phosphate at His- in a cascade leading to phosphorylation of the carbohydrate. Thus, the presence of phosphorylated HPr indicates the absence of a PTS sugar. In contrast, when glucose is present, the level of phosphorylated HPr is very low. Glucose, however, activates an HPr kinase that phosphorylates a different HPr residue, Ser-. Phosphorylation of HPr at Ser- indicates the presence of glucose. The CcpA protein binds to HPr. The complex then acts as a transcriptional regulator of the catabolite-sensitive genes.

The gal Operon: DNA Looping with a Little Help from Hu

The gal operon of E. coli consists of three structural genes, galE, galT, and galK, transcribed from two overlapping promoters upstream from galE, PG1 and PG2. Regulation of this operon is complex because aside from being involved with the utilization of galactose as a carbon source, in the absence of galactose the galE gene product is required to convert UDP-glucose to UDP-galactose, a direct precursor for cell wall biosynthesis. While transcription from both promoters is inducible by galactose, it is imperative that a constant basal level of galE gene product be maintained even in the absence of galactose. The gal operon is also a catabolite-repressible operon. When cAMP levels are high the CRP– cAMP complex binds to

the – region, promoting PG1 transcription but inhibiting transcription from PG2. When grown on glucose, however, where cAMP levels are low, transcription can occur from PG2 assuring a basal level of gal enzymes.

Both of the gal promoters are negatively controlled by the galR gene product, a member of the LacI family of repressors. The galR locus is unlinked to the gal operon. There are two operator regions to which the repressor binds: an extragenic operator, located at – from the PG1 transcription start site, and an intragenic operator, located at position + from S1 start. A galR repressor dimer binds independently to OE and OI. For repression to occur, the two DNA-bound GalR dimers must interact with each other, forming a tetramer that engineers the DNA into a repression loop. However, GalR dimers only weakly interact. In order to stabilize that interaction, the histone-like protein Hu binds to a specific site between the two operators. The DNA loop sequesters PG1 and PG2 from RNA polymerase access. Some access must occur, however, since, as mentioned above, basal levels of the gal enzymes are synthesized even under repressed conditions [7]–[9].

Attenuation by TRAP in Bacillus subtilis. In the discussion of the E. coli tryptophan operon, the concept of attenuation involving ribosome stalling was introduced. However, there are other ways a cell can utilize attenuation that do not involve ribosome stalling. The first is an RNA-binding protein-dependent attenuationsystem that regulates the formation of competing mRNA stem structures in a 5′ untranslated region. In Bacillus, a protein called TRAP forms an -member ring structure that can bind tryptophan. Tryptophan-activated TRAP binds to closely spaced AG repeats present in the nascent trpEDCFBA leader transcript. TRAP binding prevents formation of an antiterminator structure in the leader region, which leaves an overlapping Rho- independent terminator free to form. Thus, TRAP binding promotes transcription termination before RNA polymerase reaches the structural genes. In the absence of TRAP, the antiterminator structure prevents formation of the terminator, resulting in transcriptional readthrough into the trp structural genes.

The pyrBI Strategy. In E. coli or Salmonella enterica, the de novo synthesis of UMP, the precursor of all pyrimidine nucleotides, is catalyzed by six enzymes encoded by six unlinked genes and operons. One operon within this system is pyrBI, which codes for the catalytic and regulatory subunits of aspartate transcarbamylase. The operon is negatively regulated over 0-fold by intracellular UTP concentration. Attenuation in this system involves RNA polymerase pausing rather than ribosome stalling. As with the tryptophan operon, regulation depends on the degree of coupling between transcription and translation. This permits RNA polymerase to transcribe into the pyrBI structural genes. However, when UTP levels are high, RNA polymerase undergoes only a weak pause, then quickly releases to transcribe the attenuator before the ribosome has caught up. Therefore, the transcription termination loop forms, terminating transcription before RNA polymerase has entered structural genes [10]–[12].

CONCLUSION

It may seem that attenuation is a very costly and inefficient regulatory system for the cell.Why would nature provide a regulatory mechanism that involves the synthesis of nonfunctional polypeptides or transcripts? Remember that all regulatory systems require information and so of necessity require the expenditure of energy. The energetic cost of attenuation may be less than that required to synthesize a larger trans-acting regulatory protein. A clear advantage of attenuation is that it uses information present in the nascent RNA transcript. This is a strategy that quickly and directly translates what is sensed into action. Within the pyrBI regulatory region there is an RNA polymerase pause site flanked by U-rich areas. When cellular UTP levels are low, RNA polymerase undergoes a lengthy pause

as it tries to find UTPs to extend the message. This strong pause allows the translating ribosome to translate up to the stalled RNA polymerase. When the polymerase finally escapes the pause region and reaches the transcriptional attenuator region, the ρ-independent terminator hairpin cannot form due to the close proximity of the ribosome.

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CHAPTER 14

MICROBIAL PHYSIOLOGY IN THE GENOMIC ERA: A REVOLUTIONARY TALE

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ABSTRACT:

The field of microbial physiology is undergoing a dramatic revolution. This reformation is the direct result of three technological achievements: the personal computer, the Internet, and rapid DNA sequencing techniques. In the past, examining the physiology of a microorganism was a long, painstaking process. Even now, years after initiating the analysis of the common intestinal microorganism Escherichia coli, we are still far from having a completely, integrated picture of its biochemistry and genetics. Today, however, many organisms that have proven almost intractable to scientific inquiry have had much of their genetics and physiology laid bare. Examples include Rickettsia prowazekii and Mycobacterium leprae, neither of which can grow outside of a living host. We now know with some certainty what biochemical pathways they have, how they make energy, what possible virulence proteins are in their pathogenic arsenal, and how they relate evolutionarily to other bacteria. All this has come about by modern sequencing techniques that have allowed entire genomes to be decoded in relatively short periods of time and because powerful sequence analysis software has been created that identifies open reading frames and promoter sequences, and compares new sequences with those already known.

KEYWORDS:

Microbial Physiology, Microorganisms, Sequencing Techniques, Biochemistry.

INTRODUCTION

Comparing the amino acid sequence of an ORF of unknown function with all other known protein sequences identifies those proteins with sequences and motifs similar to what is present in the ORF. This analysis provides considerable insight as to the possible function of the predicted ORF in the cell without ever having conducted a single, true biochemical experiment! The personal computer and Internet have fueled this renaissance of microbial physiology by allowing unfettered sharing of this information among scientists around the world. This chapter briefly describes the basic tools molecular biologists use to examine DNA sequences, gene expression, DNAprotein interactions, and protein protein interactions. Initially, however, we discuss how the sequence of a complete genome is obtained and the means by which that knowledge can be used to study microbial physiology and evolution [1]–[3].

Cloning a Genome

Every quest to sequence a new genome begins by deconstructing the bacterial chromosome into small overlapping fragments. First, a plasmid or phage vector is chosen and cut with a restriction enzyme. Chromosomal DNA from the subject organism is extracted, digested with the same restriction enzyme, and cloned into the cutvector. Following ligation with DNA ligase, the clones are transformed into E. coli. This is known as “shotgun” cloning as opposed

to a directed cloning strategy that requires the careful identification of individual DNA fragments encoding a specific gene of interest. The result of shotgun cloning is a random clone library that can be quickly sequenced.

DNA Sequencing

Once a random clone library is constructed, DNA sequencing efforts can begin. As illustrated, a single strand from each clone is used as a template to synthesize a series of fragments, each of which is only one base longer than the previous fragment. The trick is to label the last base synthesized on each fragment with a fluorescent tag and to do it in such a way that a different colored tag is used for each of the four nucleotidespolymerases only synthesize DNA by adding a new nucleotide to a preexisiting 3′ hydroxyl group of a growing strand. The primer molecule allows DNA sequencing to begin at a predetermined, fixed point in each clone. Generating different-sized. Fragments while specifically labeling the last nucleotide incorporated into each fragment is accomplished by including small amounts of fluorescently tagged, dideoxy versions of each nucleotide to the DNA synthesis reaction mix, which also contains normaldeoxynucleotides. The key to the method is that dideoxy nucleotides lack both the 2′ and 3′ hydroxyl groups. So, if DNA polymerase chooses to add a dideoxy nucleotide to an elongating chain, the chain cannot extend any further because there is no 3′ hydroxyl primer. Because each dideoxy base is tagged differently, the last base in that fragment, and, thus, the fragment itself, is specifically labeled.

To read the sequence, the mixture of different-sized and labeled DNA fragments are separated by size via polyacrylamide gel electrophoresis, usually conducted in a capillary tube. The overall negative charge on DNA causes all fragments to travel toward the positive pole, and the sieving action of the polyacrylamide matrix allows smaller DNA fragments to travel faster than larger fragments. The result is a parade of fragments progressively moving down the gel, with each fragment being exactly one base longer than the one before it. An optical laser positioned toward the end of the gel will excite the fluorescent tag at the end of each fragment, and the color emitted is read as a specific base.

Because shotgun cloning makes fragments with overlapping ends, computer analysis of the many DNA sequences will identify and assemble the overlapping fragments into contigs. With some finishing gap filling, the contigs are assembled into a complete genome ready for annotation. Annotation is the process whereby potential genes, known as open reading frames, are identified by looking for telltale sequence signatures such as translational start codons in proximity to potential ribosome-binding sites. Then the ORF sequence and the predicted protein product are compared to the existing database to look for similarities with known genes and proteins. Based on what is found, the ORF may be annotated as having a potential function.

The Institute for Genomic Research published the first complete microbial genomic sequence, that of Haemophilus influenzae, in. Since then, over genomes have been sequenced and many more are in progress [4]–[6].

Web Science: Internet Tools for DNA Sequence Analysis

Once you get the DNA sequence for a gene you are studying, what do you do with it? The first stop these days is the World Wide Web, where you can gain amazing insight into the possible function of your gene, even if it has never been sequenced before. How is this level of prognostication possible? It all boils down to hard work and evolution. Before complete genome sequencing efforts became formalized, scientists had already identified the sequences of a large number of genes as well as the functions associated with them. These sequence-

function databases have become indispensable in biology because they can be used to assign signature sequences that predict functional motifs. Processes such as gene duplications within an organism, horizontal gene transfers between species, and the mutational divergence of genes are considered to have played major roles in shaping evolution, contributing to the rapid diversification of enzymatically catalyzed reactions and providing material for the invention of new enzymatic activities. These evolutionary relationships are why genomics is possible and serve as the basis for genomic terminology.

Two genes that appear homologous in sequence have likely evolved from a single ancestral sequence. Two terms have been used to classify the types of homology. Orthologous genes are homologs in two different organismsthat evolved from a common ancestral gene. They may or may not retain ancestral function. So, the gene encoding σ - in E. coli is orthologous to the σ - gene in other species, even species not closely related to E. coli. Paralogous genes are those whose evolution reflects gene duplications. For example, the gene encoding σ - is a paralog of the gene encoding σ -. One gene arose as a duplication of the other, followed by mutational divergence to change in this case, promoter selectivity.

As noted, the first stop once a sequence is in hand is the Web. Major Internet repositories for gene sequences include EMBL and Embank. These databases can be accessed via a number of Web sites such as through the National Institutes of Health home page or the European Bioinformatics Institute home page. Upon sequencing part or all of a gene, the researcher can enter these sites, and, through programs such as BLAST or FASTA, can compare their query DNA sequence, or deduced protein sequence, to all the known DNA or protein sequences. These programs use statistical calculations to identify significant sequence matches between the query sequence and the sequences in the database. If homology is found over a major portion of a known gene/gene product in another organism and if the function of that homolog is known, the putative function of the query gene can be cautiously predicted. Many times only a portion of the query sequence is homologous to a known gene. This may be due to the presence of a common cofactor-binding site such as ATP. This can be analyzed with another program found on the Internet that screens sequences for known motifs.

Virtual expeditions to the Internet sites listed in Table 4-1 will reveal a wealth of computer analysis tools including those that predict isoelectric points, molecular weights, three-dimensional structures, and proteolytic peptide patterns of deduced proteins, as well as those that reveal potential DNA regulatory sites, promoters, and so on. A particularly intriguing reference is the KEGG site, which graphically illustrates the biochemical systems predicted to be present in any microorganism whose genome has been sequenced. To see how this works, go to this site, open KEGG, and under “Pathway Information” click on “Metabolic Pathways.” Click on the metabolic system of interest. The reference pathway for this system will appear. In the “Go to” window, select the organism you want to query. The reference pathway will reappear but with colored boxes highlighting the enzymes predicted to be present in that organism based on the genome sequence. Clicking on a box reveals the enzyme name with amino acid and DNA sequences from that organism. A short tour around this site will underscore how extensively genomics and the Internet have transformed the field of microbial physiology.Genomics has provided some interesting insights into niche selection and species survival. For example, two pathogens, H. influenzae and Mycoplasma pneumoniae, both infect the respiratory tract, yet their strategies for acquiring solutes are distinct.

Gene Replacement

One step toward confirming a predicted function is to mutate the gene in the organism by what used to be called reverse genetics that is, identify the gene, then make the mutation rather than the other way around. A variety of techniques can be used, but most methods rely on delivering a mutated gene into the organism in such a way that easily selects recombination and replacement of the resident gene. One method, suicide mutagenesis, involves deleting an internal portion of the gene you wish to mutate and placing the deleted gene into a suicide vector. In this example, a drug cassette encoding chloramphenicol resistance has replaced the deleted section of gene. A suicide vector is an antibiotic-resistant plasmid that only replicates in cells containing a specific replication protein. The only way the plasmid can convey drug resistance on a cell missing the plasmid-specific replication protein is if the plasmid recombines.

Gene Arrays

Knowing how the cell coordinates all of its biochemical activities will be a major step toward understanding what constitutes life. Unfortunately, a method that would simultaneously examine all the biochemical and structural facets of cell physiology does not yet exist. However, there are powerful techniques available such as gene arrays and two-dimensional separation of cell proteins that provide global macromolecular “snapshots” of the cell. These procedures allow us to view how tweaking one biochemical system impacts the synthesis of all the other systems in the cell.

The first technique, gene array technology, allows us to view gene expression at the whole genome level. The expression of every gene can be monitored simultaneously using a DNA microchip. The basic idea is to attach DNA sequences derived from each gene in an organism to a solid support surface in a way that will allow for rapid hybridization to a fluorescently labeled pool of DNA. DNA sequences from individual genes occupy separate spots arranged as a grid on the chip. The entire chip is only about 1 or 2 square inches. Next, the RNA from a cell culture is rapidly extracted and converted into complimentary DNA using reverse transcriptase. By incorporating fluorescently tagged nucleotides into the reaction mix, each cDNA molecule is labeled. Individual cDNA molecules will bind to specific locations on the chip that contain their original encoding gene. Laser-scanning and fluorescent- detection devices read the chip surface, and computer analysis reveals which genes were expressed in the original sample and which were not.

Direct comparison of gene expression patterns produced by one strain grown under two different conditions can be made using gene array analysis. This is achieved by labeling the cDNA derived from cells grown under different conditions with different fluorescent markers, which is done during the synthesis of cDNA by incorporating different fluorescently tagged dTTP solutions into the reaction mixes. A single array is then used to probe a mixture of the two differentially labeled cDNA preparations. If a specific gene is expressed under only one condition, the corresponding spot on the array will fluoresce one color because only the RNA extract from cells grown in that condition will have that species of RNA. If a second gene is expressed only when cells are grown under the other condition, its spot on the array will exhibit the other color. When a composite image of the two scans is made via computer, spots corresponding to genes expressed equally under both conditions will exhibit a third color , which is a blend of the first two colors. This type of analysis can be used to examine the global effects of growth conditions, stresses, or mutations on gene expression.

DISCUSSION

Mutant Hunts

The classical approach used to expose the details of a biochemical pathway requires the presence of a selectable phenotype, such as an ability to grow on a carbohydrate. Mutants that have lost this phenotype are then sought. Once a mutant is found, the biochemical step in the pathway can be explored and the encoding gene can be mapped, identified, cloned, and sequenced.It is not unusual for a mutant hunt to screen ,0 individual mutagenized colonies and only find a handful of mutants in the particular system under study. This is why the selection phenotype is so important. If there is a positive selection phenotype, thousands of mutants can be screened on a single plate. In the analog example, only mutants that fail to transport or use the amino acid analog will grow and form colonies on a plate containing that analog. In looking for mutants defective in carbohydrate fermentation, mutant colonies that fail to ferment the test sugar will appear white among many nonmutated red colonies [7]–[9].

In the absence of positive selection, the investigator must resort to negative selection screens that are more labor intensive. For example, the phenotype of an E. coli mutant that cannot make the amino acid alanine will only grow on a defined medium if that medium contains alanine. To screen for ala mutants,0 random mutants are inoculated onto 0 rich medium plates in grids of mutants per plate. Each master plate is then replicated to two agar plates containing defined media, one with and one without alanine. Replicating colonies from master to test plates involves making an imprint of the colonies from the master plate onto sterile velveteen pads and using that pad to “stamp” the new plates. The new plates are inspected after allowing the patches time to grow. An ala mutant will be seen as a colony patch that grows on defined media containing alanine but will not grow on the same media lacking alanine.

More sophisticated mutant hunts in which individual cells rather than whole colonies can be screened are now possible with the discovery of fluorescent-activated cell sorting. To appreciate this technique, imagine making a gene fusion between a gene encoding green fluorescent protein and a gene that is only expressed under conditions where the cell will not grow and cannot form colonies. You would like to find mutants that do not express this gene at pH 4.5. To find these mutants, the gfp fusion strain can be mutated, a pool of mutants grown at normal pH, adjusted to pH 4.5 to allow induction, and a sample taken and passed through a FACs machine. The cells are passed single file through an orifice where instrumentation reads whether a cell is fluorescing. Simultaneously, a charge is placed on the fluorescing cells. Then charged and uncharged cells can be separated, and the mutant cells that do not express the test gene at pH 4.5 are collected and used for further study. This type of single- cell mutant hunt strategy has dramatically expanded the capability of microbial geneticists.

Transcriptional and Translational Gene Fusions

A very powerful tool for analyzing various aspects of gene expression involves fusing easily assayed reporter genes such as lacZ or gfp to host target gene promoters. Two types of fusions are typically used. Operon or transcriptional fusions are used when a promoterless lacZ reporter gene is inserted within a target gene in an orientation that places lacZ underthe control of the target gene’s promoter. Thus, whatever factors control expression from the

target gene promoter will also control the production of β-galactosidase. Since the lacZ message resulting from this fusion still contains its ribosome-binding site, the regulation observed is typically, although not always, due to transcriptional control. The second type of fusion involves inserting a reporter into a target gene that is missing both its promoter and ribosome-binding site. Not only will the messages from the target and reporter gene be fused but the truncated target gene peptide and the reporter gene peptide will be fused when inserted in the proper reading frame. In this case, anything controllingthe transcription or translation of the target gene will also control β-galactosidase levels.Construction of these fusions sometimes involves using a genetically engineered μ phage to randomly insert a reporter lacZ gene into the E. coli or Salmonella genome. Alternatively, the fusion can be constructed in vitro and transferredinto recipient cells in a manner that will promote allelic exchange of the reporter fusion for the resident gene. Once constructed, these fusions allow for monitoring of how different growth conditions or known regulators influence the transcription or translation of the gene. In addition, these fusions provide convenient phenotypes that can be exploited in mutant hunts to screen for new regulators [10]–[12].

CONCLUSION

There are numerous commonly used molecular tools for studying microbial physiology. This chapter presents only a few key methods chosen to underscore the impact that genomic and proteomic strategies have had on this field. Other techniques such as DNA footprinting or reverse transcriptase-mediated PCR have not been discussed. Details of these and many other tools of the trade are readily available in other publications such as Applied Molecular Genetics by Roger L. Miesfeld.As discussions progress in this textbook, you should begin to understand how many of the techniques covered in this chapter are used to answer important biochemical and genetic questions. Perhaps the most striking observation that can be made when comparing the present state of science to that of only or years ago is that many investigations that took decades to complete can now be accomplished in a mere fraction of that time using the current tools of genomics, molecular biology, and biochemistry.

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CHAPTER 15

DNA EXCHANGE,RECOMBINATION, MUTAGENESIS, AND REPAIR: AN ANALYSIS

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ABSTRACT:

The plasticity of bacterial genomes is nothing short of amazing! The processes of DNA exchange, replication, and repair are major contributors to gene shuffling and critical players in the evolutionary history of present-day bacteria. The molecular footprints of ancient DNA exchanges between diverse genetic species are evident in most, if not all, of the bacteria for which complete DNA sequences are known. In addition to their evolutionary significance, these processes are crucial for bacterial survival in natural environments where chemical insults constantly threaten to damage DNA and destroy chromosome integrity. The goal of this chapter is to describe these processes, ultimately placing them in the context of evolution and survival. Genetic recombination is the production of new combinations of genes derived from two different parental cells. Several processes have been described by which prokaryotic cells can exchange genetic information to yield recombinants. They are conjugation, transformation, and transduction. Conjugation requires phys- ical contact between two cells of opposite mating type, resulting in the transfer of DNA from the donor cell to the recipient cell.

KEYWORDS:

DNA Exchange, Bacterial Growth,Recombination, Mutagenesis, Microbial Physiology.

INTRODUCTION

The transfer of cell-free DNA into a recipient cell is called transformation, whereas transduction involves bacteriophage-mediated transfer of genetic information from a donor cell to a recip- ient cell.In the following section, genetic exchange and DNA recombination processes are considered in detail. Often, only a small portion of the donor cell genome is transferred to the recipient cell. The recipient cell temporarily becomes a partial diploid for the short region of homology between the transferred DNA and the DNA of the recipient chromosome. The ultimate fate of the donated DNA, whether it becomes incorporated into the recipient genome by recombination processes, degraded by nucleases , or maintained as a stable extrachromosomal fragment, occupies a considerable portion of the ensuing discussion [1]–[3].

Plasmids

Bacteria are sexually active organisms! This ability to transfer DNA through cell– cell contact was first discovered in the s using Escherichia coli. Transfer of genetic information between different E. coli strains was found to depend on the presence in some cells of a small “extra” chromosome called F factor. F factor encodes the proteins necessary for the sexual process and is discussed in detail in this chapter. Subsequent to the discovery of F, many other extrachromosomal DNA elements, called plasmids, were discovered. All plasmids share some common features. They are generally double-stranded, closed circular DNA

molecules capable of autonomous replication. One exception to circularity is the linear plasmids found in the Lyme disease bacterium Borrelia burgdorferi.Some plasmids, called episomes, commonly integrate into the bacterial chromosome. F factor is one example of an episome. The size of plasmids can range from 1 to kb. Compare this to the size of the E. coli bacterial chromosome, which is approximately kb. A plasmid that can mediate its own transfer to a new strain is called a conjugative plasmid, whereas one that cannot is referred to as nonconjugative. Plasmids that have no known identifiable function other than self-replication are often referred to as cryptic plasmids. However, they are cryptic only because we have not been clever enough to elucidate their true function.

It is important to examine and understand plasmids because of the enormous role they play in biotechnology and medicine. Plasmids are the workhorses of recombinant DNA technology, serving as the vehicles by which individual genes from diverse organisms can be maintained separate from their genomic origins. In addition, many plasmids harbor antibiotic resistance genes. Promiscuous transfer of these plasmids among diverse bacterial species has led to the rapid proliferation of antibiotic-resistant disease-causing microorganisms.

Partitioning

Why have plasmids been maintained throughout evolution? One reason is that they often provide a selective advantage to organisms that harbor them, such as toxins to kill off competing organisms or resistance genes to fend off medical antibiotics. On a more basic level, the reason plasmids persist in a population is due to their ability to partition. Partitioning assures that after replication each daughter cell gets a copy of the plasmid. For plasmids present in high copy numbers, random diffusion may be enough to get at least one copy of the plasmid to each daughter cell. However, random segregation of low-copy-number plas- mids would likely mean that, following cell division, one of the daughter cells would not receive a plasmid. The plasmid would eventually be diluted from the population. Consequently, regulated partitioning mechanisms are essential for these plasmids. The mechanism used for partitioning differs depending on the plasmid.

Partitioning, especially of low-copy-number plasmids such as F factor and P1, usually involves genes called par organized on plasmids as gene cassettes. Almost all plasmid-encoded par loci consist of three components: a cis- acting centromere-like site and two trans-acting proteins that are usually called Par A and B, which form a nucleoprotein complex at the centromere-like site. Cis-acting means that, for a plasmid to replicate, the DNA sequence in question must be part of that plasmid. It does not encode a diffusible product. In contrast, a trans-acting replication gene encodes a protein that does diffuse through the cytoplasm. Thus, the gene does not have to reside on the plasmid to help that plasmid replicate.

The upstream gene in a typical par operon encodes an ATPase essential to the DNA segregation process. The downstream gene encodes the protein that actually binds to the centromere. The organization of the partitioning loci in F factor and P1 are the same, but the genes are called par and sop, respectively. ParB/SopB proteins bind as dimers to the downstream parS/sopS centromere-like regions. ParA /SopA then bind through protein–protein interactions with the B proteins.Genetic fusions between the Par proteins and green fluorescent protein from the jellyfish Aequorea victoria have been used to visualize the location and movement of plasmids in the cell during segregation. The data suggest that the plasmids are intrinsically located at midcell where they replicate. After replication, the daughter plasmids pair with ParA and B proteins at the parC/sopC site, then move from midcell to quarter-cell positions, ensuring each daughter cell receives a plasmid copy. This

movement presumably requires interaction with a host-encoded mitotic-like apparatus. What comprises that apparatus is not known.

Incompatibility

Incompatibility is a property of plasmids that explains why two very similar yet distinct plasmids might not be maintained in the same cell. Two plasmids that share some regulatory aspects of their replication are said to be incompatible. For example, if two different plasmids produce similar repressors for replication initiation, then the repressor of one could regulate the replication of the other and vice versa. The choice as to which plasmid will actually replicate is random. So, in any given cell, copies of one plasmid type could outnumber copies of a second plasmid. Because of this incongruity, cell division could result in a daughter cell that only contains one of the two plasmid types. This is called segregational incompatibility

Nonconjugative, Mobilizable Plasmids

Many plasmids that are nonconjugative by themselves nevertheless possess a system that will allow conjugal transfer when present in the same cell as a conjugative plasmid. The colicin E1 plasmid is an example of a nonconjugative, mobilizable plasmid. It has a dnaA-independent origin of replication but contains a site called bom that functions much like oriT of the F factor. While some ColE1 genes are required for mobilization, transfer will not occur unless some functions are provided by F factor. Not all conjugative plasmids will fill this role, however. Where members of the incompatibility groups IncF, IncI, or IncP conjugative plasmids will efficiently mobilize ColE1, members of IncW will do so inefficiently

Resistance Plasmids

Many extrachromosomal elements have been recognized because of their ability to impart new genetic traits to their host cells. One important factor, resistance factor, was first recognized by the fact that organisms in which it was present were resistant to a number of chemotherapeutic agents. A single R factor may carry traits for resistance to as many as seven or more chemotherapeutic or chemical agents. R factors harbored by organisms in the normal flora of human beings or animals may be transferred to pathogenic organisms, giving rise to the sudden appearance of multiple resistant strains. There is evidence that R factors did not arise as a direct result of the widespread use of antibiotics. Individuals living in highly sequestered geographic areas who have not been subjected to antimicrobial agents possess normal bacterial flora harboring R factors. Also, organisms maintained in the lyophilized state from time periods prior to the widespread use of antimicrobial agents have been shown to carry R factors. Thus, the origin of these plasmids probably occurred in response to encounters with antimicrobials produced in the natural environment. Regardless of their origins, the fact that R factors are transferred from normal flora to pathogenic microorganisms under natural conditions generates considerable concern with respect to the widespread and indiscriminant use of antimicrobial agents that could foster the further spread of these plasmids.

Plasmids in Other Bacterial Genera

The association of resistance to chemotherapeutic agents and other genetic traits with extrachromosomal elements has been well established in the Enterobacteriaceae. It is also possible to demonstrate intergeneric transmission of plasmids among members of this closely related group of organisms. Plasmid-associated resistance factors have been well characterized in most bacteria. For example, resistance of Enterococcus hires to erythromycin and lincomycin is plasmid associated. Self-transferable plasmids encoding hemolysins and

bacteriocins as well as multiple antibiotic resistance have also been found in E. hirae. As is described in this chapter, transfer of plasmids among the streptococci appears to take place through conjugal mechanisms.

Plasmid Replication

There are three general replication mechanisms for circular plasmids: theta type, rolling circle, and strand displacement. Theta -type replication has been examined most extensively among circular plasmids of gram-negative bacteria. The mechanism involves melting of the parental strands at a plasmid origin, synthesis of a primer RNA, and then DNA synthesis by covalent extension of that primer. θ -type DNA synthesis can start from one or multiple origins, and replication can be either uni- or bidirectional, depending on the plasmid. Electron microscopy revealed these replication intermediates look like θ-shaped molecules. Initiation generally requires a plasmid-encoded Rep initiator protein that binds to a series of directly repeated DNA sequences within the origin called iterons.

Rolling circle replication is unidirectional. The current model posits that replication initiates by the plasmid-encoded Rep protein, introducing a site-specific nick on one strand of the DNA. The resulting 3′-OH end serves as a primer for leading strand synthesis involving host replication proteins. Elongation from the 3′-OH ends accompanied by displacement of the parental strand continues until the replicome reaches the reconstituted origin at which point a DNA strand transfer reaction takes place to terminate leading strand replication. The result is a double-stranded DNA molecule comprised of the parental minus strand and the newly synthesized plus strand, and a single-stranded DNA intermediate corresponding to the parental plus strand. The generation of this single strand is the hallmark of plasmids replicating by the RC mechanism. Finally, the parental strand is converted into dsDNA by the host replication machinery initiating at a second origin physically distant from the RC origin.

The best-known examples of plasmids replicating by the strand displacement mechanism are the promiscuous plasmids of the IncQ family. Replication is promoted by the joint activity of three plasmid-encoded proteins, RepA, B, C, with, respectively, 5′-3′ helicase, primase, and initiator activities. RepC binds to iteron sequences within the origin and probably with the RepA helicase, promoting exposure of two small. Palindromic sequences located on opposite strands at the origin. DNA synthesis occurs with opposite polarities from these two sites. Stem-loop structures generated by the ssi sequences are probably required for assembly of the RepB primase, an event required to initiate replication.

Although we tend to think of plasmids as circular, there are notable examples of linear plasmids. These plasmids have two basic structures. The spirochete Borrelia contains linear plasmids with a covalently closed hairpin loop at each end. This group replicates via concatameric intermediates. Linear plasmids in the gram-positive filamentous Streptomyces have a covalently bound protein attached at each end.Representatives of the second group have a protein covalently bound at their 5′ ends and replicate by a protein priming mechanism.

Plasmid Copy Number Control. Plasmids are selfish genetic elements. They must ensure that they are stably inherited despite constituting an energy burden on the host cell. In addition to partitioning systems, plasmids contain mechanisms that maintain a certain number of plasmid copies per cell. Too few copies can result in plasmid loss after division, whereas too many copies resulting from runaway replication will overburden the cell, leading to cell death. As noted earlier, plasmids can be characterized into separate groups based on the number of copies normally present in a cell. F factor is a low-copy-number plasmid whose replication is stringently controlled. ColE1, in contrast, is a high-copy-number plasmid that can be present

at to 0 copies per cell. Even though there is a wide difference in their copy number, both types of plasmids have mechanisms designed to limit and maintain copy number.

The ColE1 plasmid undergoes unidirectional replication and can replicate in the absence of de novo protein synthesis. It does this by utilizing a variety of stable host replication proteins rather than making its own. Because replication initiation of the bacterial chromosome requires de novo protein synthesis, the addition of chloramphenicol to ColE1-containing cells results in the amplification of the plasmid copies relative to the chromosome. But even though the ColE1 replication system allows for a fairly high copy number per cell, the system is regulated so as not to exceed a given number.

Addiction Modules: Plasmid Maintenance by Host Killing: The ccd Genes

In addition to the partitioning systems already described, several plasmid systems utilize genes called addiction modules to prevent the development of a plasmid-less population. Low-copy-number plasmids such as F factor, as well as R1, R0, and P1, all have the remarkable ability to kill cells that have lost the plasmid. In F, two plasmid- associated genes are responsible: ccdA and ccdB. The ccdB product is an inhibitor of cell growth and division. Its activity is countered by the unstable ccdA product. If plasmid copy number falls below a certain threshold, the loss of CcdA through degradation will unleash the killing activity of CcdB. Affected cells form filaments, stop replicating DNA, and die. This phenomenon is referred to as programmed cell death. The population has the appearance of being addicted to the plasmid. The target of CcdB is DNA gyrase. CcdB toxin, in the absence of the CcdA antidote, binds to GyrA, thereby inhibiting DNA gyrase activity. Cell death may be due to DNA cleavage that results from CcdB trapping the DNA gyrase– DNA complex in a poststrand passage intermediate. Alternatively, the DNA gyrase– CcdA– DNA complex will inhibit movement of RNA polymerase.

In addition to proteic postsegregational killing systems, there are antisense RNA- regulated systems. The role of antidote is played by a small, unstable RNA transcript that binds to toxin-encoding RNA and inhibits its translation. The best-characterized system of this type is the hok/sok locus of plasmid R1. Translation of mRNA encoding the Hok protein is regulated by sok antisense RNA. The unchecked production of Hok causes a collapse of the transmembrane potential.In the orthodox view of bacteria as single-celled organisms, programmed cell death does not make adaptive sense. However, it is becoming increasingly clear that in nature bacteria seldom behave as isolated cells. They often take on characteristics normally associated with multicellular organisms. Biofilms are one obvious example. In this context, adaptive sense can be made for sacrificing an individual cell that fails to meet the community standards required for survival of the species.

Conjugation

Conjugation involves direct cell-to-cell contact to achieve DNA transfer. For this process, certain types of extrachromosomal elements called plasmids are usually required. The prototype conjugative plasmid is the F, or fertility, factor of E. coli.Barriers to Conjugation. It has been known for some time that cells carrying an F factor are poor recipients in conjugational crosses. A related phenomenon, incompatibility, operates after an F′ element enters into a recipient cell already carrying an F factor and is expressed as the inability of the superinfecting F′ element and the resident F factor to coexist stably inthe same cell.

Two genes, traS and traT, are required for surface exclusion, with TraT being anouter membrane protein. It is believed that the TraT protein might block mating pair stabilization sites or, alternatively, might affect the synthesis of structural proteins necessary for

stabilization. If mating pair formation occurs in spite of the efforts of TraT, TraS is thought to block DNA-strand transfer, although how it does this is not known. An odd phenomenon never fully explained is that growth of

F+ cells into late stationary phase imparts a recipient ability almost equivalent to an F– cell. These cells are called F– phenocopies and are nonpiliated.Fertility Inhibition. Most conjugative plasmids transfer their DNA at a markedly reduced rate as compared with F because these plasmids possess a regulatory mechanism that normally represses their tra genes. The finO and finP products interact to form a FinOP inhibitor of tra gene expression. In contrast to these other plasmids, the F factor is finO finP + and so is naturally derepressed for conjugation. However, if another plasmid that is finO + resides in the same cell as F, then the F finP gene product and the coresident plasmid's finO gene product can interact, producing the FinOPF inhibitor. This will act as a negative regulator of the F factor's traJ, which itself is the positive regulator for the other tra genes. Consequently, the fertility of F will be inhibited. FinP is a nucleotide untranslated RNA molecule that is complementary to the untranslated leader region of traJ mRNA. FinP antisense RNA is thought to form an RNA/RNA duplex with traJ mRNA, occluding the traJ ribosome- binding site and thereby preventing translation of the TraJ protein. The FinO protein in F-like plasmids other than F stabilizes FinP antisense RNA by preventing degradation by RNAse E. This increases the in vivo concentration of the antisense RNA. In addition, FinO binds to the FinP and TraJ RNA molecules, promoting duplex formation and thereby enhancing inhibition of TraJ translation.Hfr Formation. The F factor is an example of a plasmid that can exist autonomously in a cell or can integrate into the bacterial chromosome. A cell that contains an integrated F factor is referred to as an Hfr cell.

Transduction

Transduction is the transfer of bacterial genetic markers from one cell to another mediated by a bacteriophage. There are two types of transduction: generalized and specialized.

Transduction

Generalized transduction is the phage-mediated transfer of any portion of a donor cell's genome into a second cell. The transducing viral particle contains only bacterial DNA, without phage DNA. During normal loading of nucleic acid into virus protein heads, the packaging apparatus occasionally makes a mistake, packaging chromosomal DNA into the phage rather than phage DNA. When a transducing bacteriophage binds to a bacterial cell, the donor DNA is injected into the bacterium by the phage and becomes integrated into the genome of the new cell through generalized recombination. Thus, all genes from a donor cell can potentially be transduced to a recipient cell population. Phages that can mediate generalized transduction include P1 and P.

DISCUSSION

A well-studied example of generalized transduction is that of Salmonella phage P. To understand how generalized transduction occurs, you must have some understanding of how this phage replicates and packages DNA into its head. Phage DNA in P is linear but circularly permuted and terminally redundant. Thus, the termini of a P DNA molecule contain duplicate DNA sequences, but different P molecules within a population contain different terminal sequences. This seemingly odd chromosome structure is due to the packaging method P uses to insert DNA into its head. After infecting a cell, the linear P DNA circularizes through recombination between the terminal redundant ends. The circular molecule replicates via a rolling circle type of DNA synthesis, forming a long concatemer. A concatemer is a long

DNA molecule containing multiple copies of the genome. Packaging of P DNA into empty heads initiates at a specific region in P DNA called the pac site, where the DNA is first cut, and then proceeds along the concatemer. Once the P head is full, a second, nonsequence-specific cut is made. This second cut also defines the start of packing for the next phage head. However, to assure that each head receives a complete genome, the P system packs a little more than one genome length of P DNA. This is the source of the terminal redundancy of the P genome. One consequence of packing extra DNA is that the packing start site changes for each subsequent phage DNA molecule packaged from the concatemer. Each duplicated sequence at the ends of the P genome is different for each packed P molecule [4]–[6].

When the packaging system encounters a sequence in the bacterial chromosome that is similar to a pac site, it does not distinguish this site from P pac sites. It will use this homologous site to package chromosomal DNA such that progressive packaging will generate a series of phage particles that carry different parts of the chromosome. The size of packaged DNA is approximately kb, which is about one- one hundredth the size of the Salmonella chromosome. Therefore, a given P will package the equivalent of one conjugation minute worth of chromosomal DNA.Cotransduction is the simultaneous transfer of two or more traits during the same transduction event, which enables the mapping of genes relative to each other. Cotransduction of two or more genes requires that the genes be close enough to each other on the host chromosome such that both genes can be packaged into the same phage head. The closer the two genes are to each other, the higher the probability they will be cotransduced. The only thing that could separate the two genes would be a recombinational event occurring between them. Thus, the closer the two genes are to each other, the smaller the recombinational target that could separate them. In abortive transduction, DNA that is transferred to the recipient cell does not become integrated into the genome of the recipient cell.

As with conjugation, transduction usually occurs most readily between closely related species of the same bacterial genus. This is due to the need for specific cell surface receptors for the phage. However, intergeneric transduction has been demonstrated between closely related members of the enteric group of organisms for example, between E. coli and Salmonella or Shigella species. Various genetic traits such as fermentation capabilities, antigenic structure, and resistance to chemotherapeutic agents are transducible. Keep in mind that transduction is not limited to the bacterial chromosome. Genetic information residing on plasmids may also be transferred by transduction.

Lysogenic conversion is dependent on the establishment of lysogeny between a bacteriophage and the host bacterial cell. Lysogeny occurs when a bacteriophage coexists with its bacterial host without losing it. In lysogenic conversion, a new phenotypic trait acquired by the host cell is due to a phage gene. Because the gene is part of the normal phage genome, every cell in a population that has been lysogenized acquires the genetic property. This mass conversion of the cell population distinguishes lysogenic conversion from transduction and other genetic transfer mechanisms.One of the most interesting and thoroughly investigated examples of lysogenic conversion is the relationship of lysogeny to the production of toxin by the diphtheria bacillus, Corynebacterium diphtheriae. Cells that are lysogenized by β phage are designated tox +. The ability to produce toxin is inherent in the genome of the bacteriophage rather than in any trait that may have been transduced from the originalhost cell. Thus, cells that are “cured” of the lysogenic state no longer produce toxin. The tox + gene is considered to be a part of the prophage genome; however, it is not essential for any known phage function and may be modified or eliminated without any effect on β phage replication. The production of erythrogenic toxin by members of the group A

streptococci and toxins produced by Clostridium botulinum types C and D have also been shown to be the result of lysogenization by bacteriophage.

Integrons

One of the consequences of the microbial genome sequencing era is the realization that bacteria are promiscuous gene swappers. This is not restricted to passing antibiotic resistance genes around. There are numerous unexplained resemblances between DNAs of evolutionarily distinct species. Genetic elements called integrons are the reason for this high level of gene trafficking. Integrons are elements that encode a site-specific recombination system that recognizes and captures mobile gene cassettes. Integrons include an integrase and an adjacent recombination site.

A glimpse into how this type of gene trafficking occurs comes from studies with Vibrio cholerae, the cholera agent. This organism has a versatile integron acquisition system embedded in its own genome that appears capable of capturing genes serendipitously encountered from other organisms. The proposed integron contains an integrase gene and adjacent gene flanked by DNA repeats that are targets for the integrase. The integrase, however, can act on similar DNA repeats that may be found flanking genes in DNA from heterologous organisms. Vibrio may encounter this heterologous DNA as a result of conjugation or transformation. The integrase can then "capture" this new DNA by recognizing these DNA repeats and can integrate the heterologous gene at one of Vibrio's own DNA repeats. Although not proven, there is plenty of evidence that many pathogens have acquired new virulence genes using this strategy.

Mutagenesis

During growth of an organism, DNA can become damaged by a variety of conditions. Any heritable change in the nucleotide sequence of a gene is called a mutation regardless of whether there is any observable change in the characteristics of the organism. We will now discuss the various mechanisms by which mutations are introduced and repaired. You may be surprised to learn that it is not usually a chemical agent that causes heritable mutations but rather the bacterial attempt to repair chemically damaged DNA.But before delving into the molecular details of this process, the terminology of mutations must be defined. For example, a bacterial strain that contains all of the genetic information required to grow on a minimal salts medium is called wild-type or prototrophic, whereas a mutant strain requiring one or more additional nutrients is auxotrophic. Mutations themselves come in a variety of different forms. A change in a single base is a point mutation.

A point mutation can change a specific codon, resulting in an incorrect amino acid being incorporated into a protein. The result is a missense mutation. The codon change could also result in a translational stop codon being inserted into the middle of a gene. Since a mutation of this sort does not code for an amino acid, it is a nonsense mutation. A missense mutation allows the formation of a complete polypeptide, whereas a nonsense mutation results in an incomplete protein. Nonsense mutations, if they occur in an operon involving several genes transcribed from a single promoter, may have polar effects on the expression of genes downstream from the mutation. A mutation of this type is a polar mutation.

Ordinarily, you would not expect a translational stop codon to interfere with the transcription or translation of downstream members of an operon, since each gene member of the operon has its own ribosome-binding site. The key to understanding polar mutations is that some nonsense mutations will cause premature transcription termination, lowering the amount of downstream message produced and available for translation. Premature transcription

termination occurs when the nontranslated mRNA downstream from the nonsense codon possesses a secondary structure that mimics transcription termination signals.If a process causes the removal of a series of bases in a sequence, the result is a deletion mutation. Likewise, the addition of extra bases into a sequence is an addition or insertion mutation. Both additions and deletions can result in changing the translational reading frame, causing all of the amino acids situated downstream of the mutation to be incorrect. The offending mutation in this event is a frameshift mutation [7]–[9].

CONCLUSION

Insomecircumstances,weshouldrealizethattheantimutagenicrepair mechanismsarenotadvantageous.Environmentalstressresponsesareoftencharacterized by the potential for genetic diversification in cell populations by mutation.Thehypermutablestatewould bean example. An alternative modelis thatgrowth ofcells on carbohydrates like glucose limits expression of error-prone repair pathways viacatabolite repression. Thus, growth on carbohydrates that do not repress the error-pronesystems will increase mutation rate and the chance that the defect will be corrected.Whateverthetruemechanism,wemaynolongerbeabletoviewmutationsandselectionsas entirelyseparateprocesses

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CHAPTER 16

A COMPREHENIVE REVIEW OF BACTERIOPHAGE GENETICS

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ABSTRACT:

Even after nearly 0 years of study, bacterial viruses continue to play an important role in the development of bacterial genetics. Their study has been instrumental in revealing many important biological processes applicable to prokaryotic and eukaryotic organisms alike. For example, T4 and φX4 have been important tools used to reveal cardinal features of the DNA replication fork. Likewise, λ phage was integral to studies dealing with the initiation of DNA replication. Interchangeable σ factors were discovered using T4 while λ proved central to our understanding of transcriptional repressors and activators. Another phage we discuss, μ, has taught us much regarding DNA rearrangements. As you will see, bacteriophages remain critically important research tools. For example, the λ family of phages has enjoyed a resurgent interest in the genomic era. The availability of complete genome sequences has revealed a pivotal role for members of this phage family in the development of E. coli virulence.

KEYWORDS:

Bacteria, Bacterial Growth,Bacteriophage, DNA, Microbial Physiology.

INTRODUCTION

The genomes of many pathogens contain entire sections, called pathogenicity islands, that encode virulence genes with an AT content different from the rest of the chromosome [1]–[3].The first step toward understanding bacteriophages is to learn something about their classification. However, characterization and classification of the bacteriophage viruses poses many problems. From a practical point of view, they can be distinguished on the basis of their natural host, host range, and other similar characteristics. In addition, they can be characterized on the basis of their RNA or DNA content. Each phage generally contains only one kind of nucleic acid. Structural symmetry and susceptibility or resistance to ether and other solvents and to other chemical agents also provide additional criteria for classification. There are over phage types that comprise phage families.

Bacteriophages range in size from to 0 nm. Large phages are barely within the realm of resolution of the light microscope. For visualization of any details of viral structure, the electron microscope is essential. The nucleic acid may be either double stranded, single stranded, circular, or linear. Morphologically, phages generally display a considerable degree of geometric symmetry. They generally have a head or capsid composed of identical protein subunits called capsomeres. The nucleic acid core is housed within the capsid. Some bacteriophages have a prominent tail structure. The capsid structure may assume an icosahedral form. The entire infectious unit is generally referred to as a virion. The smallest phages contain

Nucleic acid with a molecular weight on the order of 1 × 6 daltons. This relatively short-chain nucleic acid can obviously only code for a smallnumber of genes. These genes, of

necessity, must encode information that governs the formation of the basic viral subunits. The presence of such a small number of genes reflects the degree to which viruses are dependent on the host cell.

Each species of microorganism is susceptible to a limited subset of viruses. Put another way, each phage has a specific host range of susceptible microorganisms that it can infect. For example, a hypothetical phage A might only infect one strain of E. coli while phage B could infect many different strains of E. coli. Phage A is viewed as having a narrow host range relative to that of phage B. Host range depends on the presence of specific viral receptors on the host cell surface. These receptors are usually composed of specific carbohydrate groups on lipopolysaccharides of cell surface structures. Some viruses, such as M, attach to sex pili and are referred to as male-specific phages. The salient point here is that receptors are actually normal cellular proteins with a specific function that are co-opted by the phage [4]–[6].

Two types of infection cycles may occur following the initial infection. One is a virulent or lytic infection that ends in lysis and death of the host cell. The other type of infection is called lysogenic, which may be quite unapparent since the host cell does not die. In fact, the general appearance and activity of the lysogenized host cell may not be altered in any overt manner. A lytic type of phage only undergoes virulent infection. Temperate phages, on the other hand, can undergo either lytic or lysogenic infection.Bacteriophage infections of bacteria follow a characteristic pattern. The growth curve of bacteriophages, sometimes referred to as the one-step growth curve. Once a population of bacterial cells has been inoculated with a given number of bacteriophages, the number of detectable infectious particles rapidly decreases. This stage is termed the eclipse phase and represents that portion of the time following infection during which bacteriophages cannot be detected either in the culture medium or within the cell. The overall period encompassing adsorption and eclipse is referred to as the latent period. After the latent period has been completed, infectious viruses begin to be released from the cell. The average number of infectious bacteriophage particles released per cell is referred to as the burst size.

Bacteriophage

Bacteriophages are viruses that parasitize bacteria. Bacteriophages were jointly discovered by Frederick Twort in England and by Felix d'Herelle at the Pasteur Institute in France. Felix d'Herelle coined the term "Bacteriophage". Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture. They are commonly referred as "phage". Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. They occur widely in nature and can readily be isolated from feces and sewage. There are at least distinct groups of bacteriophages, which are very diverse structurally and genetically.

1. T-even phages such as T2, T4 and T6 that infect E.coli
2. Temperate phages such as lambda and mu
3. Spherical phages with single stranded DNA such as PhiX4
4. Filamentous phages with single stranded DNA such as M
5. RNA phages such as Qbeta

Composition

Depending upon the phage, the nucleic acid can be either DNA or RNA but not both. The nucleic acids of phages often contain unusual or modified bases, which protect phage nucleic

acid from nucleases that break down host nucleic acids during phage infection. Simple phages may have only 3-5 genes while complex phages may have over 0 genes. Certain phages are known have single stranded DNA as their nucleic acid.

Morphology

Most phages range in size from -0 nm in length. T4 is among the largest phages; it is approximately 0 nm long and -0 nm wide. All phages contain a head structure, which can vary in size and shape. Some are icosahedral others are filamentous.The head encloses nucleic acid and acts as the protective covering. Some phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. T4 tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it.The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers.

Life Cycle

Adsorption: The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers. Phages attach to specific receptors on the bacterial cell such as proteins on the outer surface of the bacterium, LPS, pili, and lipoprotein. This process is reversible. One or more of the components of the base plate mediates irreversible binding of phage to a bacterium.

Penetration

The irreversible binding of the phage to the bacterium results in the contraction of the sheath and the hollow tail fiber is pushed through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope. Nucleic acid from the head passes through the hollow tail and enters the bacterial cell. The remainder of the phage remains on the outside of the bacterium as “ghost”. Even a non-susceptible bacterium can be artificially infected by injecting phage DNA by a process known as transfection.Depending on the life cycle, phages can either by lytic or lysogenic. While lytic phages kill the cells they infect, temperate phages establish a persistent infection of the cell without killing it. In lytic cycle the subsequent steps are synthesis of phage components, assembly, maturation and release.

Lytic Cycle

Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle. Soon after the nucleic acid is injected, the phage cycle is said to be in eclipse period. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell. This phase is devoted to synthesis of phage components and their assembly into mature phage particles.

DISCUSSION

The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA's and proteins are made. In some cases the early phage proteins actually degrade the host chromosome. Structural proteins that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized. Nucleic acid is then packaged inside

the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation. In Lysis and Release Phase the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium. It is believed that phage enzymes weaken the cell wall of bacteria. The number of particles released per infected bacteria may be as high as. The average yield of phages per infected bacterial cell is known as burst size [7]–[9].

Global human health is currently being impacted by the problem of growing antibiotic resistance. Multidrug-resistant illnesses are becoming more common, making them more difficult to cure, increasing morbidity and mortality, and posing significant financial losses. The situation has become more complicated as a result of the slow discovery of new antibiotic compounds, which has compelled scientists to consider and develop antibiotic alternatives. from lytic bacteriophages have been employed in the Eastern world from their first deployment more than a century ago, this has led to the resurrection of bacteriophages as a potent alternative.

The present evaluation offers a detailed story about the significant facets of bacteriophages to aid researchers and physicians in enhancing bacteriophages as a more potent, secure, and affordable therapeutic alternative. By examining the most recent case reports along with the impacts on the microbiome of the human gastrointestinal tract, it condenses the prerequisite essential requirements of phage therapy, the function of phage biobank, along with the details of immune responses reported while using bacteriophages in clinical trials/compassionate grounds. In addition to therapeutic therapy, this review also covers the potential of bacteria as a means of biocontrol against food-borne illnesses in the food industry and aquaculture. The main obstacles are covered in the final section, along with the potential applications of phage therapy and phage-mediated biocontrols in the future. Figure 1 interaction of bacteriophage nucleic acid with the toll like receptors (TLR).

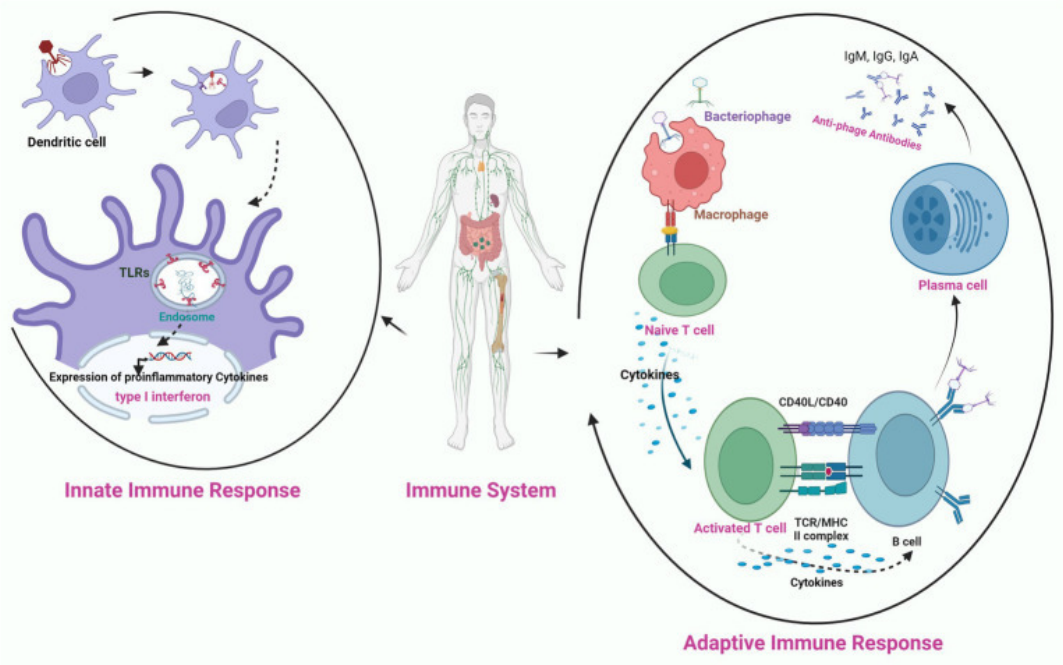

Figure 1: Interaction of bacteriophage nucleic acid with the toll like receptors (TLR) [PubMed]

Lysogenic Cycle

Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a dormant state in the cell. In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. This integrated state of phage DNA is termed prophage. This process is known as lysogeny and the bacteria harboring prophage are called lysogenic bacteria. Since the prophage contains genes, it can confer new properties to the bacteria. When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell. This process is known as lysogenic conversion or phage conversion.Significance of lysogenic conversion includes:

1. Lysogenic phages have been shown to carry genes that can modify the Salmonella O antigen.
2. Toxin production by Corynebacterium diphtheriae is mediated by a gene carried by a beta phage. Only those strains that have been converted by lysogeny are pathogenic.
3. Clostridium botulinum, a causative agent of food poisoning, makes several different toxins, 2 of which are actually encoded by prophage genomes.
4. Lysogenised bacteria are resistant to superinfection by same or related phages. This is known as superinfection immunity.

Phage Genetics

The transfer of genetic elements from one bacterium to another by a bacteriophage is termed as transduction. Transduction can be generalized or specialized. The generalized transduction is seen in lytic cycle where segments of bacterial DNA are packaged inside phage capsid instead of phage DNA. When such phages infect new bacterial cells, the bacterial DNA is injected inside. This piece of DNA may then transfer genes to the host chromosome by recombination. Any bacterial gene may be transferred in generalized transduction. Generalized transduction is usually seen in temperate phages that undergo lytic cycle. Only those genes that are adjacent to the prophage are transferred in specialized transduction [10]–[12].

Significance of Bacteriophages

1. Transduction is responsible for transfer of drug resistance, especially in Staphylococci
2. Lysogenic conversion is responsible for acquisition of new characteristics
3. Random insertion into bacterial chromosome can induce insertional mutation
4. Epidemiological typing of bacteria
5. Lambda phage is a model system for the study of latent infection of mammalian cells by retroviruses
6. Phages are used extensively in genetic engineering where they serve as cloning vectors.
7. Libraries of genes and monoclonal antibodies are maintained in phages
8. They are responsible for natural removal of bacteria from water bodies.

CONCLUSION

The DNA flanking these islands often contain remnants of viral genes, like scars marking how the organism may have acquired these genes. In addition to their evolutionary significance, the unique recombination systems of λ have been exploited to simplify prokaryotic and eukaryotic genetic engineering strategies. Even more remarkable is the renewed debate over an old idea: the potential therapeutic use of bacterial viruses as antimicrobial agents. Clearly, phage genetics has shaped modern biology and continues to be a critical factor in its development**.** The lysogenic state of a bacterium can get terminated

anytime when it exposed to adverse conditions. This process is called induction. Conditions that favor the termination of the lysogenic state include: desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals, etc. The separated phage DNA then initiates lytic cycle resulting in cell lysis and releases of phages. Such phages are then capable of infecting new susceptible cells and render them lysogenic.

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CHAPTER 17

THE E. COLI PARADIGM: A MODEL ORGANISM FOR MICROBIOLOGICAL RESEARCH

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ABSTRACT:

Life processes transform materials available from the environment into cell components. Organic materials are converted to carbon skeletons for monomer and polymer synthesis, as well as being used to supply energy. Microbes synthesize monomers in the proportions needed for growth. This is possible through the regulation of the reactions of anabolism and catabolism. With a few exceptions, microbial ecosystems are oligotrophic with a limited availability of nutrients, the raw materials used for biosynthesis. Furthermore, nutrients are not usually found in balanced concentra- tions while the organisms have to compete with each other for available nutrients. The use of Escherichia coli (E. coli) as a model organism in numerous scientific domains, such as bacterial physiology, genetics, molecular genetics, and biotechnology, is known as the "E. coli paradigm." Since it can grow quickly, has genetics that are well understood, and is simple to manipulate, the Gram-negative bacterium E. coli is commonly used as a model organism. E. coli is a diverse bacterial species that can cause intestinal or extraintestinal infections in humans and a variety of animal hosts. It includes dangerous variants as well as harmless commensal strains.

KEYWORDS:

E. coli Paradigm, Bacterial Growth,Organism, Life Processes, Microbial Physiology.

INTRODUCTION

Unlike animals and plants, unicellular microbial cells are more directly coupled to their environment, which changes continuously. Many of these changes are stressful so organisms have evolved to cope with this situation. They regulate their metabolism to adapt to the ever-changing environment. Since almost all biological reactions are catalyzed by enzymes, metabolism is regulated by controlling the synthesis of enzymes and their activity. Metabolic regulation through the dynamic interactions between DNA or RNA and the regulatory apparatus employed determine major characteristics of organisms. In this chap- ter, different mechanisms of metabolic regulation are discussed in terms of enzyme synthesis through transcription and translation and enzyme activity modulation. The rate of biological reactions catalyzed by enzymes is determined by the concentration and activity of the enzymes. Various mechan- isms regulating the synthesis of individual enzymes are discussed here before multigene regulation is considered [1]–[3].

The Escherichia Coli Paradigm

Microbial physiology is an enormous discipline encompassing the study of thousands of different microorganisms. It is, of course, foolhardy to try to convey all that is known on this topic within the confines of one book. However, a solid foundation can be built using a limited number of organisms to illustrate key concepts of the field. This text helps set the foundation for further inquiry into microbial physiology and genetics. The gram-negative

organism Escherichia coli is used as the paradigm. Other organisms that provide significant counterexamples to the paradigm or alternative strategies to accomplish a similar biochemical goal are also included. In this chapter we paint a broad portrait of the microbial cell with special focus on E. coli. Our objective here is to offer a point of confluence where the student can return periodically to view how one aspect of physiology might relate to another. Detailed treatment of each topic is provided in later chapters.

Cell Structure

As any beginning student of microbiology knows, bacteria come in three basic models: spherical, rod, and spiral. They do not possess a membrane-bound nucleus as do eukaryotic microorganisms; therefore, they are prokaryotic. In addition to these basic types of bacteria, there are other more specialized forms described as budding, sheathed, and mycelial.

The Cell Surface

The interface between the microbial cell and its external environment is the cell surface. It protects the cell interior from external hazards and maintains the integrity of the cell as a discrete entity. Although it must be steadfast in fulfilling these functions, it must also enable transport of large molecules into and out of the cell. These large molecules include carbohydrates, vitamins, amino acids, and nucleosides, as well as proteins exported to the exterior of the cell. The structure and composition of different cell surfaces can vary considerably depending on the organism.

Cell Wall. In, the Danish investigator Christian Gram devised a differential stain based on the ability of certain bacterial cells to retain the dye crystal violet after decoloration with % ethanol. Cells that retained the stain were called gram positive. Subsequent studies have shown that this fortuitous discovery distinguished two fundamentally different types of bacterial cells. The surface of gram-negative cells is much more complex than that of gram-positive cells. The gram-positive cell surface has two major structures: the cell wall and the cell membrane. The cell wall of gram-positive cells is composed of multiple layers of peptidoglycan, which is a linear polymer of alternating units of N-acetylglucosamine and N-acetylmuramic acid. A short peptide chain is attached to muramic acid. A common feature in bacterial cell walls is cross-bridging between the peptide chains. In a gram-positive organism such as Staphylococcus aureus, the cross-bridging between adjacent peptides may be close to 0%. By contrast, the frequency of cross-bridging in Escherichia coli may be as low as %. Other components for example, lipoteichoic acid are synthesized at the membrane surface and may extend through the peptidoglycan layer to the outer surface.

The peptidoglycan layer of a gram-negative cell is generally a single monolayer. An outer membrane surrounding the gram-negative cell is composed of phospholipids, lipopolysaccharides, enzymes, and other proteins, including lipoproteins. The space between this outer membrane and the inner membrane is referred to as the periplasmic space. It may be traversed at several points by various enzymes and other proteins.

Membranes

The cytoplasmic membrane of both gram-positive and gram-negative cells is a lipid bilayer composed of phospholipids, glycolipids, and a variety of proteins. The proteins in the cytoplasmic membrane may extend through its entire thickness. Some of these proteins provide structural support to the membrane while others function in the transport of sugars, amino acids, and other metabolites.

The outer membrane of gram-negative cells contains a relatively high content of lipopolysaccharides. These lipid-containing components represent one of the most important identifying features of gram-negative cells: the O antigens, which are formed by the external polysaccharide chains of the lipopolysaccharide. This lipid- containing component also displays endotoxin activity that is, it is responsible for the shock observed in severe infections caused by gram-negative organisms. Bacterial cell surfaces also contain specific carbohydrate or protein receptor sites for the attachment of bacteriophages, which are viruses that infect bacteria. Once attached to these receptor sites, the bacteriophage can initiate invasion of the cell.

Gram-positive and gram-negative cells have somewhat different strategies for transporting materials across the membrane and into the cell. The cytoplasmic membrane of gram-positive organisms has immediate access to media components. However, chemicals and nutrients must first traverse the outer membrane of gram- negative organisms before encountering the cytoplasmic membrane. Gram-negative cells have pores formed by protein triplets in their outer membrane that will permit passage of fairly large molecules into the periplasmic space. Subsequent transport across the inner or cytoplasmic membrane is similar in both gram-positive and gram- negative cells. Capsules. Some bacterial cells produce a capsule or a slime layer of material external to the cell. Capsules are composed of either polysaccharides or polymers of amino acids called polypeptides. The capsule of Streptococcus pneumoniae type III is composed of glucose and glucuronic acid in alternating β-1, 3- and β-1, 4- linkages.

Organs of Locomotion

Many microorganisms are motile that is, able to move from place to place in a concerted manner especially in an aqueous environment. In the case of bacteria, this motility is accomplished by means of simple strands of protein woven into helical organelles called flagella. The bacterial flagellum is attached at the cell surface by means of a basal body. The basal body contains a motor that turns the flagellum, which propels the organism through the liquid environment.

Pili or Fimbriae

Many bacteria possess external structures that are shorter and more rigid than flagella. These structures have been termed pili or fimbriae. These appendages also appear to arise from a basal body or granule located either within the cytoplasmic membrane or in the cytoplasm immediately beneath the membrane. Generalized or common pili play a role in cellular adhesion to surfaces or to host cells.

Ribosomes

The cytoplasm of all cells has a fine granular appearance observed in many electron micrographs. The ribosome orchestrates the polymerization of amino acids into proteins. At higher magnification under the electron microscope the ribosome particles are spherical. In properly prepared specimens the ribosomes are observed as collections or chains held together on a single messenger RNA molecule and are referred to as polyribosomes or simply polysomes.The more or less spherical ribosome particle, when examined by sucrose gradient sedimentation, has been found to have a svedberg coefficient of S. The prokaryotic ribosome may be separated into two lower-molecular-weight components: one of S and another of S. Only the complete S particle functions in polypeptide synthesis. By comparison, the ribosomes of eukaryotic cells are associated with the endoplasmic reticulum, are larger, and are composed of S and S subunits. The function of both S and S ribosomes in protein synthesis is identical. Curiously, eukaryotic mitochondria characteristically display S

ribosomes not the s particles that you would expect because mitochondria probably evolved from endosymbiotic prokaryotic cells, a hypothesis supported by extensive analyses comparing bacterial and mitochondrial genomes.

Synthesis of Dna, Rna, And Protein

The chromosome of E. coli is a single, circular, double-stranded DNA molecule whose nucleotide sequence encodes all the information required for cell growth and structure. The major molecular events required for propagating the species start with the chromosome and include DNA replication, transcription, and translation. In bacteria, replication involves the accurate duplication of chromosomal DNA and the formation of two daughter cells by binary fission. In binary fission the cell grows until a certain mass-to-DNA ratio is achieved, at which point new DNA is synthesized and a centrally located cross-wall is constructed that will ultimately separate the two daughter cells.

A simplified view of DNA replication in E. coli is shown in the diagram. The double-stranded DNA molecule unwinds from a specific starting point. The new DNA is synthesized opposite each strand. The enzyme involved in replication uses a parent strand as a template, placing adenine residues opposite thymine, and cytosine residues opposite guanine [4]–[6]. New DNA is synthesized in both directions from the origin and continues until both replication forks meet at the terminus 0◦ from the origin. At this point, cell division proceeds with cross-wall formation occurring between the two newly synthesized chromosomes

The genetic information contained within DNA is processed in two steps to produce various proteins. The enzyme RNA polymerase first locates the beginning of a gene. This area of the chromosome then undergoes a localized unwinding, allowing RNA polymerase to transcribe RNA from the DNA template. Before the RNA — called messenger RNA is completely transcribed, a ribosome will attach to the beginning of the message.As already noted, the ribosome contains of two subunits, S and S, each composed of special ribosomal proteins and ribosomal ribonucleic acids. rRNA molecules do not, by themselves, code for any protein but form the architectural scaffolding that directs assembly of the proteins to form a ribosome. The ribosome translates mRNA into protein by reading three nucleotides as a specific amino acid. Each amino acid used by the ribosome must first be attached to an adaptor or transfer RNA molecule specific for that amino acid. tRNA containing an attached amino acid is referred to as a charged tRNA molecule. A part of the tRNA molecule called the anticodon will base-pair with the codon in mRNA.

When two such charged tRNA molecules simultaneously occupy adjacent sites on the ribosome, the ribosome catalyzes the formation of a peptide bond between the two amino acids.At this point, the two amino acids are attached to one tRNA while the other tRNA is uncharged and eventually released from the ribosome. The ribosome is then free to move along the message to the next codon. The process continues until the ribosome reaches the end of the message, at which point a complete protein has been formed. Notice that synthesis of the protein begins with the N-terminal amino acid and finishes with the C-terminal amino acid. Also note that the ribosome begins translating at the 5× end of the mRNA while the DNA strand encoding the mRNA is read by RNA polymerase starting at the 3× end. Although the beginning of a gene is usually called the 5× end, this doesn't refer to the strand that is actually serving as a template for RNA polymerase.

Metabolic and Genetic Regulation

For a cell to grow efficiently, all the basic building blocks and all the macromolecules derived from them have to be produced in the correct proportions. With complex metabolic

pathways, it is important to understand the manner by which a microbial cell regulates the production and concentration of each product. Two common mechanisms of metabolic and genetic regulation are

1. Feedback inhibition of enzyme activity
2. Repression or induction of enzyme synthesis

In feedback inhibition, the activity of an enzyme already present in the cell is inhibited by the end product of the reaction. In genetic repression, the synthesis of an enzyme is inhibited by the end product of the reaction. Induction is similar except the substrate of a pathway stimulates synthesis of the enzyme. Hypothetical pathways illustrating these concepts are presented excessive production of intermediate B results in the inhibition of enzyme 1 activity, a phenomenon known as feedback or end-product inhibition. Likewise, an excess of end-product C may inhibit the activity of enzyme 1 by feedback inhibition.

In contrast to feedback inhibition, an excess intracellular concentration of end- product C may cause the cell to stop synthesizing enzyme 1, usually by inhibiting transcription of the genes encoding the biosynthetic enzymes. This action is referred to as genetic repression. The logic of this control is apparent when considering amino acid biosynthesis. If the cell has more than enough of a given amino acid, that amino acid will activate a repressor protein, which then blocks any further transcription of the biosynthesis genes. In contrast, substrates such as carbohydrates can stimulate the transcription of genes whose protein products consume that carbohydrate. This genetic process is called induction. Different organisms may employ quite different combinations of feedback inhibition, repression, and induction to regulate a metabolic pathway.

Having just outlined the processes of transcription, translation, and replication, it is now possible to define several genetic terms. The gene may be defined as a heritable unit of function composed of a specific sequence of purine and pyrimidine bases, which in turn determines the base sequence in an RNA molecule, and, of course, the sequence of bases in an RNA molecule specifies the sequence of amino acids incorporated into a polypeptide chain. The genotype of an organism is the sum total of all of the hereditary units of genes. The observed expression of the genetic determinants that is, the structural appearance and physiological properties of an organism is referred to as its phenotype.

An individual gene can exist in different forms as a result of nucleotide sequence changes. These alternative gene forms are referred to as alleles. Genetic material is not absolutely stable but can change or mutate. The process of change is known as mutagenesis. Altered genes are referred to as mutant alleles in contrast to the normal or wild-type alleles. Spontaneous mutations are thought to arise during replication, repair, and recombination of DNA as a result of errors made by the enzymes involved in DNA metabolism. Mutations may be increased by the activity of a number of environmental influences. Radiation in the form of x rays, ultraviolet rays, or cosmic rays may affect the chemical structure of the gene. A variety of chemicals may also give rise to mutations. Physical, chemical, or physicochemical agents capable of increasing the frequency with which mutations occur are referred to as mutagens. The resulting alterations are induced mutations in contrast to spontaneous mutations, which appear to occur at some constant frequency in the absence of intentionally applied external influences.

Since bacterial cells are haploid, mutants are usually easier to recognize because the altered character is more likely to be expressed, particularly if the environment is favorable to mutant development.The use of mutants has been a tremendous tool in the study of most, if not all, biochemical processes. Genes are usually designated by a three-letter code based on their

function. For example, genes involved in the biosynthesis of the amino acid arginine are called arg followed by an uppercase letter to indicate different arg genes. A gene is always indicated by lowercase italic letters, whereas an uppercase letter in the first position indicates the gene product. At this point, we need to expose a common mistake made by many aspiring microbial geneticists concerning the interpretation of mutant phenotypes. Organisms such as

E. coli can grow on basic minimal media containing only salts, ammonia as a nitrogen source, and a carbon source such as glucose or lactose because they can use the carbon skeleton of glucose to synthesize all the building blocks necessary for macromolecular synthesis. The building blocks include amino acids, purines, pyrimidines, cofactors, and so forth. A mutant defective in one of the genes necessary to synthesize a building block will require that building block as a supplement in the minimal medium. Microorganisms also have an amazing capacity to catabolically use many different compounds as carbon sources. However, a mutation in a carbon source utilization gene does not mean it requires that carbon source. It means the mutant will not grow on medium containing that carbon source if it is the only carbon source available.The chromosome of our reference cell, E. coli, is 4,9,1 base pairs long. Gene positions on this map can be given in base pairs starting from the gene thrL, or in minutes based on the period of time required to transfer the chromosome from one cell to another by conjugation.

Chemical Synthesis

Our paradigm cell can reproduce in a minimal glucose medium once every minutes. This comprises about one trillion cells more than 0 times the human population of the planet. When calculating the concentration of a compound within the cell, it is useful to remember that there are 3 to 4 microliters of water per 1 milligram of dry weight. Our reference cell, although considered haploid, will contain two copies of the chromosome when growing rapidly. It will also contain, 0 ribosomes and a little over 2 million total molecules of protein, of which there are between and different varieties. As you might gather from these figures, the bacterial cell is extremely complex. However, the cell has developed an elegant strategy for molec- ular economy that we still struggle to understand. Some of what we have learned is discussed throughout the remaining chapters.

In just minutes an E. coli cell can make a perfect copy of itself growing on nothing more than glucose, ammonia, and some salts. How this is accomplished seems almost miraculous! All of the biochemical pathways needed to copy a cell originate from just precursor metabolites. To understand microbial physiology, you must first discover what the metabolites are and where they come from.The metabolites come from glucose or some other carbohydrate. The catabolic dissimilation of glucose not only produces them but also generates the energy needed for all the work carried out by the cell. This work includes biosynthetic reactions as well as movement, transport, and so on. A composite diagram of major pathways for carbohydrate metabolism with the metabolites highlighted. Most of them are produced by the Embden-Meyerhof route and the tricarboxylic acid cycle. Three are produced by the pentose phosphate pathway.These compounds are siphoned off from the catabolic pathways and used as starting material for the many amino acids, nucleic acid bases, and cofactors that must be produced. Subsequent chapters deal with the specifics of each pathway.

Energy

Another mission of carbohydrate metabolism is the production of energy. The most universal energy transfer compound found in living cells is adenosine triphosphate. The cell can generate ATP in two ways: by substrate-level phosphorylation in which a high-energy phosphate is transferred from a chemical compound to adenosine diphosphate during the

course of carbohydrate catabolism; or by oxidative phosphorylation in which the energy from an electrical and chemical gradient formed across the cell membrane is used to drive a membrane-bound ATP hydrolase complex to produce ATP from ADP and inorganic phosphate.The generation of an electrical and chemical gradient across the cell membrane requires a complex set of reactions in which H+ and e− are transferred from chemical intermediates of the Embden-Meyerhof and TCA cycles to a series of membrane-associated proteins called cytochromes. As the eis passed from one member of the cytochrome chain to another, the energy released

The cell does not only catabolize glucose via glycolysis. There are alternate metabolic routes available for the dissimilation of glucose. One use for alternate path- ways of carbohydrate metabolism is the generation of biosynthetic reducing power. The cofactor NAD is actually divided functionally into two separate pools. NAD is used primarily for catabolic reactions, whereas a derivative, NAD phosphate, and its reduced form, NADPH, are involved in biosynthetic reactions. The phosphoketolase pathway is necessary for the generation of the NADPH that is essential for biosynthetic reactions.

Nitrogen Assimilation

A major omission in our discussion to this point involves the considerable amount of nitrogen needed by microorganisms. Every amino acid, purine, pyrimidine, and many other chemicals in the cell include nitrogen in their structures. Since glucose does not contain any nitrogen, how do cells acquire it? Some microorganisms can fix. Atmospheric nitrogen via nitrogenase to form ammonia and then assimilate the ammonia into amino acids. Other organisms such as E. coli must start with NH4+. The assimilation of N involves the amidation of one of the key metabolites, α-ketoglutarate, to form glutamic acid. After assimilation intoglutamate, the amino nitrogen is passed on to other compounds by transamination reactions. For example, glutamate can pass its amino group to oxaloacetate to form aspartate. It can be seen that aspartate, like glutamate, is a precursor for several other amino acids. The subject of nitrogen assimilation is covered in depth.

Endospores

A few bacteria such as Bacillus and Clostridium produce specialized structures called endospores. Endospores are bodies that do not stain with ordinary dyes and appear as unstained highly refractile areas when seen under the light microscope. They provide resistance to heat, desiccation, radiation, and other environmental factors that may threaten the existence of the organism. Endospores also provide a selective advantage for survival and dissemination of the species that produce them. Under the electron microscope, spores show a well-defined multilayered exosporium, an electron-dense outer coat observed as a much darker area, and a thick inner coat. In the spore interior, the darkly stained ribosomes and the nuclear material may also be visible.

Growth

Growth of a cell is the culmination of an ordered interplay among all of the physiological activities of the cell. It is a complex process involving

1. Entrance of basic nutrients into the cell
2. Conversion of these compounds into energy and vital cell constituents
3. Replication of the chromosome
4. Increase in size and mass of the cell

5. Division of the cell into two daughter cells, each containing a copy of the genome and other vital components

Microbiologists usually consider the phenomenon of growth from the viewpoint of population increase, since most current techniques do not allow the detailed study of individual cells. A study of the increase in population implies that each cell, as it is produced, is capable of producing new progenywhere a is the number of organisms present in the original inoculum. Since the number of organisms present in the population is a function of the number 2, it becomes convenient to plot the logarithmic values rather than the actual numbers. Plotting the number of organisms present as a function of time generates a curvilinear function. Plotting the logarithm of the number, a linear function is obtained. For convenience, logarithms to the base are used. This is possible because the logarithm to the base of a number is equal to 0. times the logarithm to the base 2 of a number.

Up to this point it has been assumed that the individual generation time is the same for all cells in the population. However, in a given population, the generation times for individual cells vary, so the term doubling time encompasses the doubling time for the total population. As the cells initially experience a period of adjustment to the new environment, and there is a lag in the time required for all of the cells to divide. Actually, some of the cells in the initial inoculum may not survive this lag phase and there may be a drop in the number of viable cells. The surviving cells eventually adjust to the new environment and begin to divide at a more rapid rate. This rate will remain constant until conditions in the medium begin to deteriorate. Since plotting the cell number logarithm during this period results in a linear function, this phase of growth is referred to as the logarithmic phase or, more correctly, the exponential phase.

Continuous Culture

Usually bacteria are grown in “batch” culture in which a flask containing media is inoculated and growth is allowed to occur. This is a closed system where it is actually very difficult to manipulate growth rate. In batch cultures, growth rate is determined internally by properties of the bacteria themselves. A batch culture can be used to grow bacteria at different rates as long as the nutrient added is at a concentration that does not support maximal growth. But, to accomplish this, the cell density, and thus the cell number, will be too low for certain analyses. To grow bacteria at slow growth rates and at high cell density, a chemostat is used. In this apparatus, fresh medium containing a limiting nutrient is added from a reservoir to the culture vessel at a set rate. The volume in the culture vessel is kept constant by an overflow device that removes medium and cells at the same rate as fresh medium is added. In a chemostat, growth rate is determined externally by altering the rate-limiting nutrient added to the culture vessel. The faster the limiting nutrient is added, the faster the growth rate.

DISCUSSION

All living organisms have certain basic nutritional requirements: sources of carbon, nitrogen, energy, and essential growth factors are needed to support growth. Microorganisms vary widely in their nutritional requirements. Two main groups of organisms are classified on the basis of their ability to gain energy from certain sources and the manner in which they satisfy their carbon and nitrogen requirements for growth:

1. Lithotrophs utilize carbon dioxide as the sole source of carbon and gain energy through the oxidation of inorganic compounds or light. Inorganic nitrogen is utilized for the synthesis or organic compounds.

2.Organotrophs generally prefer organic substrates as a source of energy and carbon. Photoorganotrophs utilize light as a source of energy for the assimilation of carbon dioxide as well as organic compounds. Chemoorganotrophs utilize organic compounds for growth.

Although their nutritional requirements are remarkably simple, chemolithotrophic bacteria must be metabolically complex since they synthesize all of their cellular components and provide the energy for this activity through the oxidation of inor- ganic compounds. One fundamental characteristic of strict chemolithotrophs is that they are unable to grow on or assimilate exogenous organic compounds. Faculta- tive chemolithotrophs can utilize exogenous organic carbon sources. Chemolithotrophs possess unique mechanisms for carbon dioxide fixation such as the ribulose bisphos- phate cycle and the reductive carboxylic acid cycle. Some organotrophic organisms utilize carbon dioxide as a source of carbon, but most prefer organic carbon sources and generally cannot subsist on carbon dioxide as the sole carbon source. Organotrophs may use inorganic nitrogen, but most members of the group grow better when supplied with organic nitrogen compounds. For example,E. coli, Enterobacter aerogenes, yeasts, and molds grow luxuriantly on glucose as the only organic nutrient. Other organotrophs such as streptococci and staphylococci also exhibit specific requirements for one or more nitrogen sources as amino acids, purines, or pyrimidines.

Fatty acids are required by some organisms, particularly in the absence of certain B vitamins. Replacement of a growth factor requirement by the addition of the end product of a biosynthetic pathway in which the vitamin normally functions is referred to as a sparing action. This type of activity has been reported for many growth factors, including amino acids, purines, pyrimidines, and other organic constituents. If a vitamin can completely replace a particular organic nutrient in a defined medium, that nutrient cannot be regarded as a true growth requirement since it can be synthesized in the presence of the requisite vitamin.Although most bacterial membranes do not contain sterols, they are required in the membranes of some members of the Mycoplasmataceae. Mycoplasma require sterols for growth. Acholeplasma do not require sterols; however, they produce terpenoid compounds that function in the same capacity as sterols. Fungi contain sterols in their cell membranes but in most cases appear to be capable of synthesizing them.

Oxygen

Microorganisms that require oxygen for their energy-yielding metabolic processes are called aerobes, while those that cannot utilize oxygen for this purpose are called anaerobes. Facultative organisms are capable of using either respiratory or fermentation processes, depending on the availability of oxygen in the cultural environment. Aerobic organisms possess cytochromes and cytochrome oxidase, which are involved in the process of oxidative phosphorylation. Oxygen serves as the terminal electron acceptor in the sequence and water is one of the resultant products of respiration. Some of the oxidation– reduction enzymes interact with molecular oxygen to give rise to superoxide, hydroxyl radicals, and hydrogen peroxide, all of which are extremely toxic.

Carbon Dioxide

Many organisms are dependent on the fixation of carbon dioxide. Certain organisms thrive better if they are grown in an atmosphere containing increased carbon dioxide.Haemophilus, Neisseria, Brucella, Campylobacter, and many other bacteria require at least 5 to % carbon dioxides in the atmosphere to initiate growth, particularly on solid media. Even organisms such as E. coli use carbon dioxide to replenish intermediates in the TCA cycle that have been siphoned off as precursors for amino acid synthesis. These anapleurotic reactions include pyruvate carboxylase, phosphoenolpyruvate carboxylase, or malic enzyme [7]–[9].

Extremophiles

Microorganisms vary widely in their ability to initiate growth over certain ranges of temperature, hydrogen ion concentration, and salt concentra- tion. Organisms that function best under extreme environmental conditions are called extremophiles. Examples include bacteria found in hot springs and in the thermal vents on the ocean floor. These organisms prefer to grow at extremely high temperatures. Some microorganisms prefer to live in an acidic environment while others prefer an alkaline pH. E. coli prefers a neutral pH environment and thus is classified as neutralophilic. The ability of certain organisms to grow in extreme environments can be linked to the possession of unique membrane compositions and/or enzymes with unusual temperature or pH optima that are more suitable to their environment [10], [11].

Microbial Stress Responses

For normalophiles, meaning organisms that prefer to grow under conditions of ◦C, pH 7, and 0.9% saline, variations in pH and temperature have a marked impact on enzyme activity and, ultimately, viability. Outside their optimal parameters, enzymes function poorly or not at all, membranes become leaky, and the cell produces Compounds that damage DNA and other macromolecular structures. All of these factors contribute to cell death when the cell is exposed to suboptimal environments. However, many, if not all, microorganisms have built-in stress response systems that sense when their environment is deteriorating, such as when medium acidifies to dangerous levels. At this point, signal transduction systems perceive the stress and transmit instructions to the transcription/translation machineries to increase expression of specific proteins whose job is to protect the cell from stress. The various genetic regulatory systems and protection strategies used by the cell to survive stress are discussed[12], [13].

CONCLUSION

This chapter is a highly condensed version of the remainder of this book, provided to build a coherent picture of microbial physiology from the start. Too often textbooks present a student with excruciatingly detailed treatments of one specific topic after another without ever conveying the “big picture.” As a result, the information overload is so great that the student, lost in the details, never develops an integrated view of the cell and what makes it work. Our hope is that the framework in this chapter will be used to build a detailed understanding of microbial physiology and an appreciation of its future promise. Aerotolerant organisms generally do not produce catalase. Hence, growth of these organisms is frequently enhanced by culture on media containing blood or other natural materials that contain catalase or peroxidase activity. Organisms that do not utilize oxygen may tolerate it because they do not interact in any way with molecular oxygen and do not generate superoxide or peroxide. Anaerobic bacteria from a variety of genera are present in the normal flora of the animal and human body as well as in a number of natural habitats such as the soil, marshes, and deep lakes. A number of the more widely known genera of anaerobic organisms are listed.

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CHAPTER 18

AN ANALYSIS OF BACTERIA-SURFACE INTERACTIONS

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ABSTRACT:

A few of the significant outcomes of bacteria interacting with surfaces include the production of bioenergy, biofouling, the formation of biofilms, and the infection of plants and animals. The interactions between bacteria and surfaces often modify the expression of genes involved in cell shape and behaviour, such as those needed for motility and surface attachment. While these characteristics have drawn attention, it is still unknown how bacteria perceive surfaces and respond to them. Research on the physiology of bacteria in contact with surfaces will benefit from an understanding of these processes, which will also guide the development of novel materials that both inhibit and promote cell growth. Recent discoveries from a number of scientific and technological fields will probably serve as a roadmap for future study in this area. In this review, open research questions are highlighted along with an overview of the surfaces that have been used in bacterial investigations, analyses of surface sensing mechanisms and the effects of cell attachment, reviews of recent studies on bacteria-surface interactions, and an analysis of the surfaces used in bacterial investigations.

KEYWORDS:

Bacteria-Surface, Bacterial Growth,Interactions, behaviour, Geobiology.

INTRODUCTION

Geobiology and molecular phylogeny suggest that the beginning of microorganisms occurred between 3.5 and 3.8 billion years ago, or only one billion years after the Earth's formation. Since then, microbes have flourished, and there are now thought to be 4-6 billion of them on Earth and in almost every investigated terrestrial environment. The majority of bacteria in the biosphere live in communities that are connected to surfaces, according to a basic concept of microbiology4, and this connection has proved essential to the capacity of bacteria to effectively colonise varied environments. Surfaces contain a range of characteristics that protect cells from predators and other environmental hazards, encourage genotype conservation, and aid in the establishment of cell communities[1]–[3].

There have been investigations on bacterial adherence to surfaces for some time. PS Meadows examined how salinity influenced how marine and freshwater organisms clung to glass slides in some of the early works on this subject. 5 Early studies on attachment to biotic surfaces6-8 predominated, including studies on epithelial cells and teeth. Thereafter, interest in abiotic surfaces grew rapidly. 9- Throughout the s, less than per year of publications on bacterial adhesion to surfaces were indexed by PubMed; this number increased to 5 in. Much of the advancements made over the last ten years may be attributed to early research in this area, particularly Katsikogianni and Missirlis' excellent investigation of Staphylococcus aureus adherence to surfaces.

Positive Aspects of Germs Sticking to Surfaces

Surface adhesion is very advantageous to bacteria. Attachment to horizontal surfaces encourages bacterial development when organic material float in liquid settles, is deposited on surfaces, and increases the local nutrient level. Similar to this, increasing the substrate's surface area makes it possible for nutrients to bind to greater surface area, enabling cells to grow at nutrient concentrations that would otherwise be inadequate to support growth. Caulobacter crescentus is a unique example of a bacterium that uses surface attachment to maximise nutrient absorption. In C. crescentus, stalked cells and motile cells alternately adhere to surfaces via a polar flagellum and a protein holdfast. This phenotypic change allows cells to adapt to both nutrient-rich and nutrient-poor environments.

In addition to how surface attachment aids nutrient collection, certain bacteria directly take the cofactors and metabolites they require from the surfaces to which they are attached. When growing on metal surfaces, Shewanella and other bacterial species may use iron and magnesium as terminal electron acceptors in respiration. Extracellular organelles facilitate the easier transfer of ions between cells and surfaces. For example, Geobacter sulfurreducens uses pili to transport charges between cells and surfaces. Via the use of an outer membrane protein complex, Shewanella oneidensis establishes an electron bridge between the periplasm and the extracellular environment [4]–[6].

As bacteria stick to surfaces, biofilms often form, which serve a variety of defensive roles. Extracellular polymeric substance secreted by the cells shields biofilms that are attached to surfaces from mechanical damage and shear brought on by fluid flow. Moreover, biofilms often display resistance to antibiotic treatment. 4 A few of the mechanisms causing this resistance include the barrier function of the biofilm matrix, the presence of latent persister cells and highly resistant micro colony variants, and the activation of several antibiotic resistance genes unique to biofilms. This and other mechanisms are thoroughly examined by Mah and O'Toole.

Moreover, recent studies have shown that the resistance profiles of cells adhering to surfaces but unrelated to biofilms are similar to those of biofilm cells. This resistance phenotype does not need genomic DNA alterations as the procedure is reversible and the cells become antibiotic-responsive after separation. John et al. claim that there are two key processes by which surface attachment increases antibiotic resistance: by reducing the net negative charge on bacterial cells and by enhancing membrane stability. Gene mutations that cause similar phenotypes reduce antibiotic resistance in microorganisms. Our results show that the metabolic state of bacteria, which is a feature of bacteria throughout the stationary phase of cell growth, is affected by their attachment to surfaces and reduces their susceptibility to antibiotics. Even surface attachment alone may not be required for this trait, given that antibiotic resistance occurs at extraordinarily high cell densities. Yet adhering to surfaces is one way bacterial populations may expand to such high densities.

In addition to antibiotic resistance, biofilm cells often suffer predator defence. When exposed to protozoa, the bacterium Serratia marcescens rapidly develops surface-associated microcolonies, a preliminary step in the creation of biofilms that protect cells from the grazing of these predators. Chemical sensing systems create chemicals that are hazardous to protozoans as the S. marcescens biofilm grows, providing an extra line of defence against predators.Bacteria attached to surfaces, particularly those associated to biofilms, may become specialised in contrast to cells in other regions of the community. In Bacillus subtilis biofilms, mobile cells, EPS-producing cells, and spore-forming cells are localised in diverse locations. The inability of certain B. subtilis strains to form organised biofilms prevents them

from sporulating, suggesting that localization or specialisation are required for the production of bacterial spores. The microcolonies and biofilms that are associated with Pseudomonas aeruginosa surfaces may include similar cell groups that move differently and are more or less sensitive to antimicrobials.

Swarming, an essential adaptive action in which contact between cells and surfaces programmes morphological changes that promote cooperative behaviour, fast community development, and community movement, is preceded by surface sensing. There have been reports of swarming motility in at least several distinct bacterial taxa from various natural settings. During bacterial swarming, a number of adaptive mechanisms have been described, including decreased resistance to drugs and mutualistic interactions with fungal spores. The protein flagellin, which assembles into the flagellar filament, is expressed at varying amounts by geographically separate groups of cells in B. subtilis swarming colonies, and these cells also exhibit various cell morphologies. Similar to this, many areas of swarming communities include Proteus mirabilis cells with various cell shapes.

In bacterial communities like swarms or biofilms, cells engage in a variety of interactions with one another. Quorum sensing, a method of bacterial communication, involves the utilisation of tiny molecules as chemical messengers. In bacterial communities, where cells are closely packed together, a higher concentration of tiny chemicals that communicate between cells and cause physiological changes is made possible. In biofilms and communities adhering to surfaces, the structure of chemical gradients in close proximity to the surface facilitates the flow of chemical information. As compared to planktonic cells that are floating in fluids freely, lateral gene transfer is also improved in biofilms. Moreover, Vibrio cholerae has characteristics that support "natural competence" as a result of surface-associated proliferation. Even the proteins and lipids in their outer membranes are exchanged by myxobacteria cells found in biofilms.

Adhering to surfaces also has a number of drawbacks, such as the inhibition of motility, which is frequently brought on by a "switch" in the activation of genes involved in adhesion and motility. For instance, the same transcriptional regulator that activates genes for extracellular matrix production may also turn off genes encoding for flagella. Cells are prevented from looking for the best habitats when nutrients are scarce by inhibiting cell motility. In certain situations, bacterial cells may be able to overcome this drawback by detecting surfaces and inducing surface-associated phenotypes that promote mobility and inhibit adherence. For instance, swarming promotes cell motility, offers many of the benefits already mentioned, and acts as a mechanism for actively acquiring nutrients. As a precursor to invading the host, certain infections employ surface sensing as a trigger to upregulate virulence factors.

Bacterial Attachment Mechanisms

Before adhering to surfaces, many bacteria are freely floating in bulk fluids. Motile bacteria can live in one of three fluid regions: bulk liquid, where the cells are not affected by the surface; near-surface bulk liquid, where the cells are affected by the surface's hydrodynamics; or near-surface constrained, where the cells are affected by the surface's physicochemical and hydrodynamic effects simultaneously. Bos and colleagues have explored the significance of physicochemical factors in bacterial adherence to surfaces in great detail.

Non-motile bacteria stick to surfaces at low and moderate fluid velocities. Non-motile bacteria are carried with the flow of fluids at high fluid velocities but do not adhere. Regardless of the fluid's flow rate, motile bacteria adhere to surfaces. It's important to note that bacteria that have dysfunctional flagella attach equally to cells without flagella whereas

bacteria that are motile adhere differently only when motile cells activate their flagella. Bacteria's buoyant density, which is typically 1.–1. g mL1, causes cells suspended in bulk liquid to slowly deposit onto surfaces. Several marine bacteria's sedimentation rates have been measured, and they vary from to m h1. It's interesting to note that when E. coli cells approach the stationary phase, their buoyant density rises, allowing for a faster deposition on surfaces. Hence, populations of cells that come into touch with surfaces may be enhanced with cells that are in the stationary phase. Vibrio parahaemolyticus cells, in contrast, see a drop in buoyant density as they go through the stationary phase.

Two steps of cell attachment happen when surfaces come into touch. Initial attachment includes hydrodynamic and electrostatic interactions, is reversible, and happens quickly. The force of adhesion between germs and surfaces grows quickly during this time. The attachment of polystyrene beads to a surface exhibits a similar phenomenon, and a number of observations indicate that this phenomenon is caused by physicochemical factors, such as the loss of interfacial water, structural alterations in surface molecules, and repositioning of the cell body to maximise attachment to the surface. The majority of bacteria interact preferentially with positively charged surfaces because they have a net negative surface charge, especially early in the stationary phase of cell development. Due to charge filtering, this effect is lost in medium with high ionic strength. The contact of bacteria with surfaces during the early stages of biofilm development may be facilitated by the increase in negative charge on cell surfaces brought on by quorum sensing in E. coli.

The hydrophobic area of the outer cell wall interacts with the surface by van der Waals interactions in the second stage of attachment, which is irreversible and lasts for many hours. Moreover, a number of proteins participate in the change from reversible to irreversible cell attachment. While its mechanism is uncertain, the cytoplasmic P. aeruginosa protein SadB is necessary for this transformation. Lipopolysaccharide and pili both speed up the initial rate of cell attachment and the pace at which it transforms into an irreversible attachment in E. coli. An ABC transporter and a secreted protein are necessary for irreversible attachment in Pseudomonas fluorescens. The synthesis of EPS, which attaches cells to surfaces, also promotes irreversible adhesion.

Early discoveries that various bacteria inhabiting the same niche do not always contact with the same surfaces provided the first indication that certain bacteria may engage selectively with particular surfaces. Streptococcus salivarius has a predilection for the tongue whereas Streptococcus mutans binds to teeth but not the tongue. Bacteria contain numerous distinct kinds of extracellular organelles, such as flagella, pili, and curli fibres, that facilitate particular adhesion to surfaces. Adhesins, proteins that bind to molecules on the surface of hosts, are commonly found near the ends of these organelles. Type I pili, which attach to glycoproteins containing alpha-D-mannose, and type IV pili, which bind to phosphatidylethanolamine, are two instances of E. coli pili. A number of oral bacterial species are drawn to the proline-rich proteins in saliva. Flavobacterium johnsoniae is a very intriguing case of specialised attachment. The adhesin SprB is necessary for F. johnsoniae to move on agar. In addition to moving along the cell surface and binding to agar surfaces, SprB also causes cell motility in relation to the agar surface. Cell adhesion and motility on surfaces other than agar may be facilitated by predicted SprB homologs in the F. johnsoniae genome.

During surface colonisation, reversible cell attachment does not always result in irreversible adhesion. FimH, a pilus tip adhesin, promotes weak cell attachment in E. coli, allowing cells to roll over surfaces. FimH facilitates the binding of glycoproteins with terminal mannose residues to N-linked oligosaccharides. Shear stress affects FimH binding to mono-mannose, and a specific amount of force is necessary to transition from weak to strong adhesion. A

wild type strain of E. coli that attached poorly to mannosylated surfaces colonised the surface more quickly than a mutant strain that was only capable of robust adhesion, according to Anderson and colleagues.

Adhesion of Cells to Many Types of Materials

Many applications, such as industrial processes, water purification systems, and medical implants, are hampered by bacterial adhesion to surfaces. Despite the application of anti-fouling coatings, it has been estimated that the cost of biofouling for a single class of Navy ships is about $1 billion each year. Biofouling of metal ship hulls often starts with bacterial adherence before moving to bigger marine species. Bacterial adhesion to surfaces is tightly controlled by thermodynamics. When the surface energy of the bacterium is greater than the surface energy of the liquid in which they are floating, cells adhere more readily to hydrophilic objects. Bacteria attach more readily to hydrophobic surfaces because their surface energy is often lower than the surface energy of the liquids in which they are floating. Glass, aluminium, stainless steel, various organic polymers, and fluorinated materials like TeflonTM are just a few of the materials to which bacteria may adhere. The variety of materials used to research bacterial adherence to surfaces is summarised in Table 1 along with pertinent works in this field. In a recent detailed analysis of the materials aspects of bacterial adhesion, Banerjee et al.

Moreover, bacteria adhere to surfaces that at first discourage cellular adhesion. This process takes place as a result of the deposition of a layer of proteins, including those produced by bacteria and those present naturally in the environment, that "condition" the surface and conceal functional groups that hinder cell attachment. For making surfaces that are bacterially resistant, the creation of conditioning layers provides a difficulty. Surfaces with quaternary ammonium compounds, which are initially bactericidal before conditioning layers are produced, are a great example. Notwithstanding the difficulties caused by biofouling, research is ongoing to create surfaces that are impervious to bacterial adhesion. Controlling surface chemistry and surface structural characteristics are two methods that have been suggested for minimising bacterial adhesion. One of the most efficient chemical methods for preventing bacterial adhesion to surfaces is the use of zwitterionic surfaces, such as betaine- and silver-impregnated surfaces that progressively release silver ions. Using thermoresponsive hydrogels, like poly, is another option. This polymer experiences a phase shift above a certain temperature and exhibits a hydrophobic surface that promotes cell adhesion and proliferation. Adsorbed cells are released from the surface when the temperature is lowered because the polymer expands and provides a hydrophilic surface.

Anti-adhesive coatings have been effectively applied using polymer brushes, in which one end of a polymer is connected to a surface and the polymer chain is extended into solution.Surfaces coated with Pluronic F-7, a tri-block co-polymer of polyethylene oxide and polypropylene oxide, lower Staphylococcus aureus and Staphylococcus epidermidis' initial attachment and development rate and make it possible to remove biofilms made up of these organisms using fluid flow.The same research discovered that Pseudomonas aeruginosa adhesion and biofilm clearance were unaffected by Pluronic F-7-coated surfaces.Natural objects like shark skin and lotus leaves have repeatedly served as the inspiration for physical techniques for decreasing connection. SharkletTM technology, nanostructured surfaces with a low effective stiffness, and slick liquid-infused porous surfaces are a few examples of structured materials intended to lessen bacterial adhesion. Recently, a thorough study of these and other methods for avoiding bacterial attachment was conducted. The qualities of surfaces perceived by bacteria and the biological machinery involved in this process may be better understood if materials that inhibit cell adhesion are replaced with those that promote growth.

Ways through which bacteria perceive surfaces and surface characteristics. Spatial gradients may be sensed by cells by comparing the signal input at one time interval to the total signal at a prior time interval.

DISCUSSION

The chemotaxis system, which employs membrane-embedded receptors to determine the concentration of external small molecules and ions and affects the direction of bacterial motion, is the classic illustration of this mechanism in bacteria. Recent research has shown that under some circumstances, bacteria are able to sense spatial changes in concentration. Staphylococcus aureus cells react by localising receptors to the surface-associated area when they detect the binding of surface ligands to receptors on one side of their cell bodies. This mechanism is more about how a cell reacts to a step-function than it does an actual extracellular gradient, but it shows that bacteria are capable of differentiating between signals coming from geographically diverse groups of cell surface receptors.

The development of biofilms is an illustration of how chemical gradients affect bacteria that are surface-associated. As cells cling to surfaces, the thin layer of fluid in between the cell body and the surface may trap ions and tiny molecules, creating a microenvironment with unique characteristics from the bulk liquid. The pH of fluid at the surface falls below the pH of the bulk liquid phase when E. coli sticks to surfaces, and this pH difference lasts for at least h. The connection between pH sensing and cell-surface reactions may be made possible by the Cpx two-component system. The expression and assembly of pili in E. coli are likewise regulated by this mechanism. Moreover, the proton motive force is amplified and cellular bioenergetics are directly impacted by the drop in pH of fluids in close proximity to surface-associated cells. 6 It has been shown that cells that stick to surfaces create 2–5 times more ATP per cell than cells that do not adhere [7]–[9].

Similar to pH, when cells adhere to substrates, the osmolality of liquids around surfaces likewise changes quickly. OmpA is used by gram-negative bacteria to detect osmolality, and this mechanism affects the transcription of other genes. Via the Cpx pathway, OmpA inhibits the synthesis of cellulose and promotes the development of biofilm in E. coli. 8 Although CpxA-mediated pH sensing by OmpA has been found as a needed component for biofilm formation in Sodalis glossinidius, an endosymbiont of the tsetse fly, this function for OmpA may be conserved across bacteria. EnvZ activates OmpR, a cytoplasmic response regulator, which deregulates the expression of genes that produce pili. Changes in osmolality promote OmpR autophosphorylation and activation while stabilising the cytoplasmic domain of EnvZ conformations.

Sensing may start before bacteria come into touch with the surface because certain surfaces produce soluble chemicals that attract bacteria. The second-most common natural polysaccharide on Earth, chitin is a homopolymer made of N-acetyl-D-glucosamine. GlcNAc monomers and oligomers, which are chemoattractants of V. cholerae, are produced by the breakdown of chitin. Type IV MSHA pili are used by V. cholerae cells to detect and migrate towards the source of GlcNAc and bind to chitin surfaces. Using the protein ChiS2, cells detect the presence of GlcNAc oligomers and in response, upregulate the transcription of multiple genes, including those that produce chitinolytic enzymes and parts of type IV pili.

Surfaces and the close proximity of other connected cells limit bacteria that adhere to surfaces physically. These physical restrictions may prevent motile bacterial cells' flagella from rotating, which changes the physiology of the cell. Cells feel the effects of surfaces,

viscous liquids, and anti-flagella antibodies on the rotation of the flagella on V. parahaemolyticus, S. marcescens, and P. mirabilis5, which results in surface-related morphological alterations that are detailed below. While the precise mechanism by which the flagellar signal is sent is unknown, it has been proposed that Umo proteins, which are membrane-associated, are important in P. mirabilis.In surface sensing, other membrane-based processes are also important. For instance, the outer membrane lipoprotein NlpE is necessary for the stimulation of the Cpx signalling pathway following E. coli surface adherence. 9 Cell interaction with a hydrophobic surface has the potential to cause damage to the outer membrane, activate NlpE, and open the Cpx pathway, which reacts to stress on the envelope. The Cpx pathway also contributes to the development of biofilms, as was previously mentioned.

Phenotypic Modifications Brought On By Cell Interaction With Surfaces

Many cellular alterations are triggered by surface sensing. Many morphological modifications that promote adhesion to surfaces are present. For instance, S. aureus cells preferentially localise fibronectin-binding protein when exposed to fibronectin-displaying surfaces. A reduction in OmpX in the membrane during E. coli cell attachment causes an increase in EPS generation and changes in antibiotic sensitivity. Quorum sensing modifies the charge on E. coli cells during biofilm development, which affects the bacteria' ability to adhere to surfaces with electrostatically attractive charges. Only when a cell comes into touch with the host tissues do certain organisms, including Helicobacter pylori2 and S. aureus, develop the organelles needed for attachment. P. aeruginosa activates the Wsp system in response to surfaces, which is made up of a group of proteins with similarity to the chemotaxis system and that dynamically locate in cells. The chemoreceptor-like protein WspA recognises surfaces and transmits a signal to the other Wsp proteins, which phosphorylates WspR at the end. Phosphorylated WspR catalyses the production of cyclic-di-GMP, which is involved in the creation of biofilms, the inhibition of P. aeruginosa swarming motility, and the control of a number of motility- or attachment-related bacterial systems. C-di-GMP influences the function of the transcription factor FleQ in P. aeruginosa.

In reaction to the presence of host surfaces, pathogenic bacteria typically uncouple their division from growth and form filaments.During urinary tract infections, the filamentation of uropathogenic E. coli makes it easier for cells to evade the host immune response. When coming into touch with plant host tissues, Agrobacterium tumefaciens also forms filaments.Swarming, a process related to surface sensing, also involves filamentation. A vast range of modifications to the global transcription of bacterial genes take place during swarming. Significant morphological changes result from these modifications, including an increase in flagellar surface density and cell length. Moreover, the genes for hemolysin, protease, urease, and proteins that aid host invasion are upregulated in swarming cells [10]–[12].

CONCLUSION

It's interesting to note that surface characteristics may affect community organisation and cell shape. The marine bacterium SW5 clings to and develops on both hydrophobic and hydrophilic surfaces, but the characteristics of the surface affect how it forms communities. Cells create microcolonies, cling evenly to hydrophobic surfaces, and develop into densely packed multi-layer biofilms. Less cells adhere to hydrophilic surfaces, and modifications to cell division cause chains of cells to develop that are >0 m long. These chains entangle loosely to create biofilms that are less densely packed and more loosely organised. Inhibiting the expression of the genes necessary for EPS production is another function of FleQ, the

master regulator of flagella gene expression. Since FleQ binds to c-di-GMP, FleQ's suppression of pel gene expression is alleviated in vivo by high levels of c-di-GMP. 9 One of the best-studied methods of bacterial surface sensing is the surface-induced rise and subsequent function of c-di-GMP in P. aeruginosa, which also serves as an illustration of how physical surfaces affect bacterial biochemistry and physiology.

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CHAPTER 19

BACTERIAL EXOPOLYSACCHARIDES: FUNCTIONALITY AND PROSPECTS

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ABSTRACT:

Bacteria of all taxa produce a variety of structural, useful, and valuable polysaccharides, which are then secreted into the environment. These polysaccharides, which go by the name exopolysaccharides, may either be homopolymeric or heteropolymeric in composition and have a range of high molecular weights. Exopolysaccharides' material qualities have changed the industrial and medicinal fields thanks to their wide range of potential uses. These applications span a wide range of industries, including pharmacology, nutraceuticals, functional foods, cosmetics, insecticides, and herbicides, while future uses might include anticoagulants, thrombolytics, immunomodulators, cancer treatments, and bioflocculants. This review offers fundamental knowledge on the physiologic and morphologic activities of bacterial exopolysaccharides as well as their applications and future possibilities in the medical and industrial fields due to the wide range of uses for these substances.

KEYWORDS:

Bacteria, Exopolysaccharides,Functionality, Microbiology, Prospects.

INTRODUCTION

Using a range of simple to complicated substrates, bacteria build a variety of biopolymers with a wide range of chemical characteristics. Some of these biopolymers have the same biological purpose, whereas others are unique to various taxa and have different biological purposes. Biopolymers may either be intracellular or extracellular depending on where in the cell they are found. The range of extracellular biopolymers is vast and can be divided into four major classes: polysaccharides, inorganic polyanhydrides, polyesters, and polyamides. These classes have been collectively referred to as extracellular polymeric substances, slime, and microcapsular polysaccharides, among others. Intracellular biopolymers are few and have very limited applications. They perform a variety of tasks, such as cell adhesion to surfaces, prokaryote migration in groundwater, defence against predatory protozoa and white blood cells, defence against the enduring effects of drying or desiccation in some soil bacteria, defence against attack by antimicrobial agents of plant or animal origin, and community life in biofilm. According to the sea ice microbial community and bacteria from other marine settings, Nichols et al. and Junge et al. indicate functions such as cryoprotection for growth at low temperatures and high salinity.

Again, the categorization of extracellular biopolymers is based on their position in relation to the cell, with polysaccharide components being the most prevalent. They are components of teichoic acids and provide structural and defensive functions at the cell wall. They may be entirely expelled into the environment as slime, a covalently attached cohesive layer, or a capsule when they are outside the cell. Dextran is an excellent example of this type of capsules; they often act as adherents of cells to surfaces and may be overproduced when there

is an oversupply of carbohydrates to become stores of glucose for future metabolism. Yet, the difference between an extracellular polymeric material that is loosely connected and one that is not rests in their structural and functional connection to the cell.

The discovery of bacterial biopolymers' use to man as a result of technological improvement has secured a wide range of industrial and medicinal applications. Some of these bacterial exopolysaccharides are naturally biocompatible and appear to be non-toxic, which has led to their use in a variety of medical applications including scaffolds or matrices in tissue engineering, drug delivery, and wound dressing. This makes them more desirable than polysaccharides derived from plants and microalgae. Certain biopolymers are suitable for use in controlled medication release and tissue replacement because they progressively disintegrate in vivo. This review outlines recent developments in our understanding of the functional characteristics of bacterial exopolysaccharides, as well as their potential uses in the medical and industrial fields.

The Functional and Morphologic Characteristics of Bacterial Exopolysaccharide

The discovery of an exopolysaccharide in wine that would later become known as dextran, and the identification of the prokaryote responsible for its synthesis as Leuconostoc mesenteriodes, marked the beginning of the history of bacterial exopolysaccharides. Other exoplysaccharides that have been found throughout time include cellulose, alginate, and xanthan. The employment of bacteriolytic enzymes and radioactive labelling of precursors for biosynthetic investigations, as a result of scientific advancements, allowed for the elucidation of certain information about the metabolic pathways for the production of biopolymers. One example is the depolymerization of the polysaccharide using a viral-borne endoglycanase, which led to the production of alditol, which was then used to elucidate the capsular polysaccharide from Klebsiella K. The structure and functional functions of bacterial exopolysaccharides increasingly became clear as a result.

The word "capsule" refers to layers of surface-associated covalently bonded polysaccharides that are anatomically positioned as the outermost coating of bacterial cells. They give bacterial colonies grown on laboratory agar medium a mucoid look. More than eighty distinct varieties of Escherichia coli alone have been identified, reflecting the structural variety demonstrated. The structure and content of capsular polysaccharides are highly strain dependant. Nonetheless, certain kinds are shared by bacteria from several taxa. A prime example of this is the sialic acid-containing capsules seen in E. coli K1 and Neisseria meningitidis B. Some bacteria are taxonomically categorised according to the polysaccharides that make up their caps. E. coli is an example of this, as the organism is classified according to its O-antigen, which comes from the cell wall, H-antigen, which comes from the flagella, and K-antigen, which comes from the capsule. L, B, and A groups are further split into the K-antigen. During the colonisation of tissues, capsules serve as adhesion receptors for the bacterial cells and act as a barrier to desiccation because they attract water. Moreover, the many structural variations provide tolerance to different phages and the vertebrate complement.

Exopolysaccharides

Exopolysaccharides are bacterial polysaccharides that are produced and released into the external environment or that are produced extracellularly by cell wall-anchored enzymes. Bacterial polysaccharides may be divided into groups depending on their chemical makeup, intended uses, molecular weight, and linking bonds. Exopolysaccharides may be examined based on monomeric composition after the chemical composition; as a result, homopolysaccharides and heteropolysaccharides are the two recognised groupings. Whereas

heteropolysaccharides are made up of repeated units ranging in size from disaccharides to heptasaccharides, homopolysaccharides only include one kind of monosaccharide.

Exopolysaccharide categorization is difficult, and there are times when characterization factors are used again to further distinguish between groups. This is evident in homopolysaccharides, which have been further clustered into four groups: -d-glucans, -d-glucans, fructans, and polygalactan. This grouping is based on linkage bonds and the nature of monomeric units. Contrarily, the repeating units of d-glucose, d-galactose, l-rhamnose, and, in certain cases, N-acetylglucosamine, N-acetylgalactosamine, or glucuronic acid are included in the composition of heteropolysaccharides. There are sometimes non-carbohydrate substituents such phosphate, acetyl, and glycerol. The backbone of the polymers is made up of 1,4- or 1,3-linkages as well as 1,2- or 1,6-linkages between monomeric units. The former has high stiffness, whilst the later exhibits flexible ones.

The distinctions between homopolysaccharide and heteropolysaccharide may be seen in the synthetic enzymes used and the location of synthesis, in addition to the chemical makeup and linking bonds. While homopolysaccharide syntheses need a specific substrate, like sucrose, the precursor repeating units of heteropolysaccharide are formed intracellularly, and isoprenoid glycosyl carrier lipids are involved in translocating the precursors across the membrane for subsequent extracellular polymerization. Moreover, different bacterial species create different amounts of exopolysaccharides. Nevertheless, pH, temperature, incubation duration, and medium composition are among the physicochemical parameters that have a significant impact on the yield of these molecules. Contrary to homopolysaccharides, it is unclear if the chemical nature or monomeric compositions of heteropolysaccharides are altered by carbon and nitrogen sources [1]–[3].

Exopolysaccharides have also been divided into functional groups; as a result, Flemming et al. established seven categories for exopolysaccharides: constructive or structural, sorptive, surface-active, active, instructive, redox-active, and nutritional exopolysaccharides. Nevertheless, the categorization is not entirely comprehensive, according to a theory put out by Flemming et al., since exopolysaccharides implicated in biocide resistance are not included. In summary, even though much has been accomplished in this sector, more work is still required. According to the aforementioned classification, neutral polysaccharides are included in the biomolecules categorised as structural exopolysaccharides because they provide architectural functions in the matrix that aid in water retention and cell protection. Exopolysaccharides with surface activity comprise molecules with amphiphilic behaviour; they have a range of chemical structures and surface characteristics, are potentially implicated in the production of biofilms, and sometimes exhibit antibacterial or antifungal capabilities. Exopolysaccharides that bind to other charged molecules involved in cell-surface interactions are called sorptive exopolysaccharides.

Significant Bacterial Polysaccharides from the Ocean

A wide variety of polysaccharides produced by marine bacteria have potential uses in biotechnology, industry, and the development of cell therapy and regenerative medicine, among other things. There are many opportunities for the discovery of new polysaccharides due to the richness and variety of the marine environment, which includes deep sea hydrothermal vents, Arctic, and Antarctic Sea ice.Spirulina has been utilised for nutritional reasons and as medicinal additions without posing any health risks. Moreover, research indicate that substances mostly made of polysaccharides present in spirulina have anti-inflammatory capabilities in addition to serving other medicinal purposes. Spirulan, a sulfated polysaccharide made by Arthrospira platensis, has also been shown to suppress pulmonary

metastasis in humans as well as tumour cell adhesion and proliferation. To this purpose, approaches for the treatment of spinal cord injury including the electrospinning of spirulina biomass to porous scaffolds and nanofibers have been created. Similar to this, the marine bacterium Vibrio diabolicus generates polysaccharides that resemble hyaluronic acid and have been sold under the brand name "Hyalurift". It has been shown that the polysaccharide exhibits bone integrity repair activities.

Bacterial Biofilm Exopolysaccharides

In nature, bacteria do not live as planktonic scattered single cells as is the case in laboratory pure cultures, but rather as colonies that gather at interfaces to create poly-bacterial aggregates like mats, flocs, sludge, or biofilms. Several germs are involved in this effort;thus, bacteria are not acting alone. Our focus, however, is on bacteria and the important functions that their exopolysaccharides perform. Moreover, for the sake of clarity, we will continue to refer to microbial aggregates that amass at a solid-liquid interface and are covered in a matrix of highly hydrated extracellular biopolymers as biofilms. But free-floating microbial aggregates are not included in this description.

Exopolysaccharides of bacteria Antigen

Bacterial exopolysaccharides are strictly defined as all types of polysaccharides that are generated and released into the external environment of cells, whether they are partially or entirely separated from the surface. The principal surface antigens for slimy bacteria are polysaccharide capsular components, and their contribution to pathogenicity has received substantial study. Yet, because of the exopolysaccharides' high degree of diversity in terms of monomeric units, links, and distinctive structures, a variety of immunogenic reactions are evoked, and these antigenic characteristics are included in the serologic categorization of bacteria. This is evident in the Enterobacteriaceae family where more than one serotype of E. coli has been discovered based on the antigen of the capsular polysaccharide.

N. meningitidis, E. coli, Salmonella typhi, Staphylococcus spp., and Streptococcus spp. are examples of bacteria that exhibit capsular polysaccharide antigenicity that transcends the Gram status split. Bacterial serotyping based on capsular polysaccharides is based on the reactivity of certain antibodies, often produced in animals using reference strains of particular species, with the guilty bacteria. Due to the structural variety of polysaccharides, different types of antibody reactivities result, as shown by the several serotypes that may be identified in bacteria belonging to the same species. Table 3 lists a few microorganisms that are clinically significant, related disorders, capsule nomenclature, and the number of recognised serotypes based on capsular polysaccharides. Bacterial serotyping has been crucial in terms of epidemiology since it is a quick and easy process that is useful during epidemics. Since certain bacterial infections may only be caused by a few serotypes of particular bacterial species, it is crucial to track the distribution of the causative agents during epidemics. The invention of vaccines based on capsular polysaccharides, which have been mostly effective in preventing infectious illness, was made possible by the structural elucidation of bacterial surface polysaccharides and immunological advancements.

Expectations for Bacterial Exopolysaccharides in the Future

Exopolysaccharides in bacteria have been primarily credited with the favourable impacts on human health that they have on the creation of functional foods. Probiotics, which Salminen et al. define as living microbial dietary components that are beneficial to human health, are a term used to characterise some of these microorganisms. Exopolysaccharides have been linked to some of the probiotics' health-promoting properties. These exopolysaccharides have

been shown to have antitumor, antiulcer, immunomodulatory, antiviral, and cholesterol-lowering properties, among other health advantages. Due to the unique rheological effects it has on goods, Lactobacillus lactis subsp. cremoris is widely used in the dairy industry to produce yoghurt; nevertheless, it is also believed that this particular bacterium has certain health-promoting qualities.

Additional Lactobacillus strains that have been implicated include Lactobacillus helveticus, Lactobacillus rhamnosus, and Lactobacillus delbrueckii subsp. bulgaricus. Moreover, research by Martin et al. indicates that certain bacterial exopolysaccharides alone or in conjugates have the potential to operate as highly effective somnogens, so switching to a natural sleep aid with no negative side effects will remove the need for xenobiotics [4]–[6].Although bacterial exopolysaccharide applications are found in a variety of fields, including industry, health, and the environment, their use in the flocculation process will be a significant step towards environmental responsibility and the promotion of good health, particularly in municipal and wastewater treatment procedures. Inorganic aluminium salts like poly-aluminum chloride and aluminium sulphate, as well as organic synthetic polymers like polyacrylamide derivatives and polyethylene imine, have been used to flocculate suspended particles in water treatment facilities.

There is a need for secure substitutes since these flocculants have been demonstrated to have harmful health consequences such neurotoxicity, carcinogenicity, and Alzheimer's disease. The examination of various bacterial products' flocculating characteristics has been motivated by this goal. Cosa et al., Piyo et al., and Mabinya et al., among others, from our group of investigations have found excellent flocculation efficiency mediated by the biopolymers generated by fresh water and marine water isolates of Virgibacillus sp. Rob, Bacillus sp. Gilbert, and Artrobacter sp. Raats. Whereas Arthrobacter sp. Raats revealed approximately% polysaccharide content, the flocculation-mediating biopolymers of Virgibacillus sp. Rob and Bacillus sp. Gilbert were constituted of saccharide moiety, since they are essentially carbohydrates. According to these results, bacterial exopolysaccharides successfully influence flocculation and may thus be used in large-scale industrial operations, particularly those involving the treatment of water and wastewater [7]–[9].

Chemotropism is the process through which living things, including individual cells like those found in bacteria and plants, expand when exposed to chemical stimuli from the environment. Whether the growth is towards the stimulus or away from the stimulus, the reaction of the organism or organism component is referred to be "positive," and vice versa. When the pollen tube grows, which always points in the direction of the ovules, this is an example of chemotropic movement. The ovary produces substances that encourage the growing pollen tube to respond positively to chemotropism, which is how pollen fertilises flowers. The roots of a plant are a good illustration of positive and negative chemotropism since they develop in the direction of helpful minerals, demonstrating positive chemotropism, and away from detrimental acids, demonstrating negative chemotropism.

DISCUSSION

It is also possible to say that the transformation of a flower into a fruit is an example of chemotropism [1], [2]. The development of individual neuronal cell axons in response to extracellular signals, which direct the growing axon to innervate the proper target tissue, is another example of chemotropic movement [3]. Another example of chemotropism is the addition of atmospheric nitrogen, often known as nitrogen fixation [4].

Chemotrophism and the Need for It

Any form of "tropism" in plant biology refers to a plant's physiological reaction to an outside stimulus. Usually, there is some kind of movement in reaction. Chemotropism is a kind of tropism in which a chemical stimulation causes the plant's organism to expand. Botanists assert that plants may exhibit both adverse and favourable chemotropisms. Research have shown that certain plants have a tendency to avoid bad soil in favour of better growth environments, which raises the possibility that the root and stem systems are capable of chemical sensing [5].

Why Maximum Yield? Chemotropism

Certain plant roots become more sensitive to possible intruders like fungus or bacteria as a result of chemotropism. Similar to this, these plants immediately multiply in locations with a lot of iron since they have an enhanced knowledge of nutritional sources. Moreover, some investigations have shown that pollen may react chemically to stimulus, which causes the pollen tube to begin adapting to outside stimuli [6], [7]. Plants may migrate in the following ways, according to botanists. Thermometer, phototropism, gravitropism, geotropism, hydrotropism, and others

Backwind Electron Flow

Microbial metabolism uses a technique called reverse electron flow. It takes energy to decrease NAD+ in chemolithotrophs that utilise an electron donor with a greater redox potential than NAD+/NADH, such as nitrite or sulphur compounds. The energy required for this process—which is the opposite of forward electron transport—is provided by consuming proton motive force, which propels electrons along an electron transport chain in the opposite direction. The energy used in reverse electron transport may, under certain circumstances, be five times larger than the energy acquired during the forward phase. This method may be used by autotrophs to provide reducing energy for inorganic carbon fixation [8].

Assimilation of Co2

Only autotrophic bacteria utilise CO2 as their single or main source of carbon, despite the fact that most microbes can repair or absorb carbon dioxide. CO2 is reduced or assimilated using a significant amount of energy. Certain autotrophic microbes get their energy via the oxidation of reduced inorganic electron donors, but often they get it by trapping light during photosynthesis. By using the Calvin cycle, reductive tricarboxylic acid cycle, hydroxypropionate cycle, or acetyl-CoA route, microorganisms may fix CO2 or transform this inorganic molecule to organic carbon and ingest it. Almost all microbial autotrophs incorporate carbon dioxide utilising the Calvin cycle, a unique metabolic route. Archaea, certain obligately anaerobic bacteria, and some microaerophilic bacteria lack this cycle, despite it being present in the majority of photosynthetic microorganisms. These microbes often use the remaining two of the aforementioned routes.

CONCLUSION

Exopolysaccharides produced by bacteria have a wide range of activities and exhibit tremendous variety. The monomeric compositions, linkage bonds, and related conjugates show some of this variation, whilst the functions may be divided into intrinsic and applied. Human use, including dairy products, cosmetics, pharmaceuticals, and other industrial and environmental applications, exhibits the inherent functions, which also include morphological, structural, and protective ones. Exopolysaccharides generated by bacteria have a wide range of uses, but in order for them to be used in human consumption, they must

either be GRAS-certified or, in the case of environmental applications like water treatment, have a practical way of neutralising harmful components. The bio-flocculation procedure serves as an example of how production costs have primarily been a limiting factor in the non-industrial use of numerous potential exopolysaccharides. Although the search for bacteria with high exopolysaccharide and exopolysaccharides conjugate yields is still ongoing, there are some suggested methods for increasing the likelihood that these compounds will be produced on a large scale and used in the field, including genetic and metabolic engineering, fermentation condition manipulation, and the exploration of inexpensive fermentation substrates. These methods are the focus of ongoing research in our group.

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CHAPTER 20

SOCIAL EVOLUTION THEORY FOR MICROORGANISMS

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ABSTRACT:

In order to engage in a variety of multicellular behaviours, including dispersal, nutrient acquisition, biofilm formation, and quorum sensing, microorganisms communicate with one another and work together. The underlying genetic regulation of these behaviours and the molecular mechanisms involved in them are being rapidly understood by microbiologists. These behaviours are also intriguing from the perspective of social evolution; why, then, do microorganisms exhibit them, given that selfish cheaters can take advantage of cooperative individuals and benefit without bearing their fair share of the burden. In this young field of sociomicrobiology, there is a lot of room for interdisciplinary research, but one barrier is the ineffective dissemination of social evolution theory to microbiologists. Here, we give a conceptual overview of the various mechanisms cooperative behaviours can be stabilised, focusing on the features that are most pertinent to microorganisms, the unique issues that microorganisms present, and the fresh perspectives that can be obtained by using evolutionary theory to study microorganisms. For scientists who study the social behaviour of microorganisms, this is an exciting time.

KEYWORDS:

Social Evolution, Bacterial Growth,Microorganisms, Theory, Multicellular Behaviours.

INTRODUCTION

Microorganisms are known to communicate with one another and work together to carry out a variety of multicellular behaviours, including dispersal, foraging, biofilm formation, "chemical warfare," and quorum sensing1-6. Interest in these behaviours is being sparked by both the behaviours themselves and the implications that arise from the fact that many of these behaviours are linked to bacterial virulence [1]–[3].Microbiologists are quickly improving their understanding of the underlying genetic regulation of these behaviours and the molecular mechanisms involved1. This literature often makes the assumption that cooperation is preferred because it benefits populations or species8,9. Nevertheless, evolutionary theory demonstrates that this approach is unworkable since the population is vulnerable to invasion by egotistical people who do not collaborate but may gain from others' collaboration. In general, one of the biggest issues with evolutionary theory is understanding cooperation. Since they allow for genetic modification and experimental evolution, microorganisms are particularly advantageous for solving this issue.

Interdisciplinary research that combines both mechanistic and evolutionary methods has a lot of promise in this field. The degree to which social evolution theory is effectively conveyed to microbiologists, however, represents a substantial limiting factor. The social evolution idea has also been subjected to a number of red herrings throughout the years, which have caused confusion and fruitless discussion. Microbiologists may save time by using the disputes that have previously occurred and been resolved in the evolutionary literature. This will help them stay away from distractions and potential causes of misunderstanding. Thankfully,

evolutionary scientists have also developed a largely coherent body of theory that can be broadly applied throughout this time. Here, we provide a summary of this theory, highlighting its features that are most pertinent to microorganisms, the unique issues they provide, and the lessons that may be learned from using evolutionary theory to understand microbes.Figure 1 microbial evolutionary medicine: from theory to clinical practice - the lancet infectious diseases.

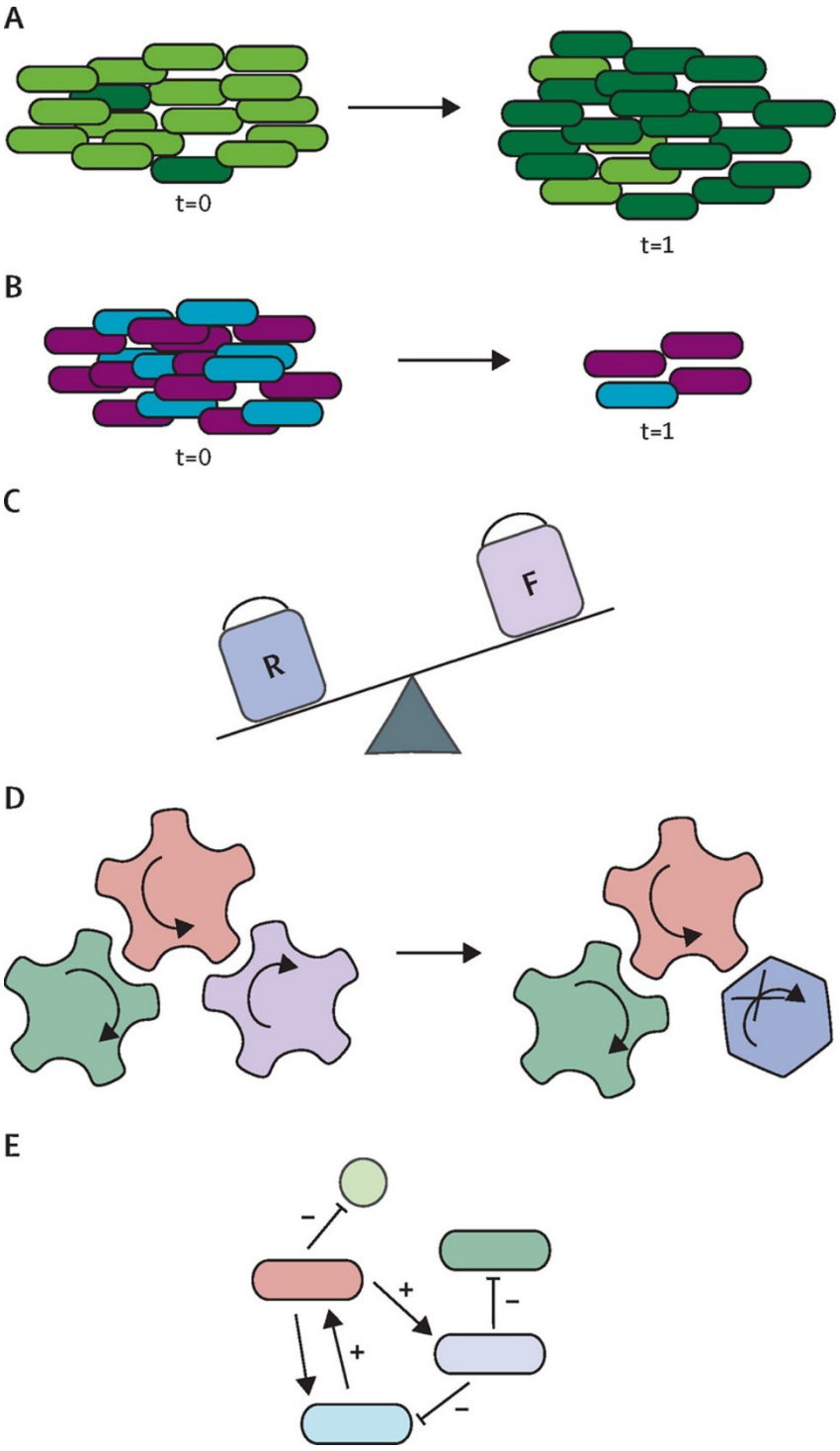

Figure 1 Microbial evolutionary medicine: from theory to clinical practice - The Lancet Infectious Diseases [Google].

The Issue of Collaboration

For evolutionary biologists, explaining cooperation is a challenge. Why would a person engage in an expensive cooperative behaviour for the benefit of others or the community? The Darwinian principle of "survival of the fittest" seems to be directly contradicted by this. Hence, cheats who do not cooperate but profit from others collaborating would obtain an advantage in the marketplace and be able to infiltrate and rule the populace. The tragedy of the commons is the name given to this issue in the disciplines of economics and human morals. The irony is that although collaboration would benefit humans as a whole, it is unstable since each person might profit by savagely pursuing their own immediate interests. Hardin demonstrated this by using the example of a shared pasture that many shepherds may use.

Even if this results in overgrazing, it is in each shepherd's best advantage to add more sheep since he reaps all the benefits of doing so while only bearing a fraction of the expense as it is split among all the shepherds. There are countless additional human examples, such as overfishing-related fish supply declines or low public support for immunisation campaigns [4]–[6].Contrary to what is often believed, the population-level benefits of collaboration do not provide a solution to this issue.Examining cooperation from the standpoint of a person or a gene may show this. It may spread among a population of cooperators even if doing so lowers the group's production if an individual can maximise its reproductive success and, thus, increase the transfer of genes to the next generation.

Defining Collaboration

How can cooperative behaviours be maintained given the issue of cooperation? There is a tonne of literature on this subject, and there are several methods to explain the various options. Our goal is to provide a categorization that is helpful for microorganisms and that microbiologists who are not experts in evolutionary theory can understand.When a behaviour is beneficial to another person, it is called cooperation. We categorise potential justifications for collaboration into two major categories. First, the cooperative behaviour has the potential to directly improve an individual's capacity for reproduction at a cost that exceeds the cost of engaging in the behaviour. The benefits of collaboration are reciprocal in this situation. We categorise this into circumstances in which people have a common interest in cooperating and circumstances in which there is a genetic mechanism for enforcing collaboration.

According to this, higher levels of altruistic cooperation should be anticipated when r or b are high and c are low. By studying how relatedness is determined by the quantity of clones in a region or patch, the influence of relatedness can be shown. At one extreme, relatedness will be great and each patch will be colonised by a cooperator or a cheater if only one clone colonises each patch. Cooperators will outcompete cheaters because cooperation promotes faster development rates. As a result, cooperation is evolutionary stable at high relatedness. On the opposite extreme, relatedness will be minimal if numerous colonies colonise each patch, and each patch may include a mix of cheaters and cooperators. In this scenario, cheaters may take advantage of and outcompete cooperators, making cooperation evolutionarily unstable. This emphasises how population advantages alone cannot account for cooperation.

As was already established, the creation of public goods has the ability to enhance fitness both directly and indirectly. This implies that if the advantages exceed the costs to the cell producing them, certain production of public goods, such as siderophores, may still be preferred while interacting with non-relatives. The relative cost and benefit of producing siderophores will change depending on several factors, including the environment and

population density. As a result, it's feasible that the creation of public commodities like siderophores may sometimes be altruistic and occasionally be advantageous to both parties. The siderophore selection experiment supports our suspicion that kin selectionwhich favours altruismwill typically be the deciding factor.

Kin discrimination and kin selection. Kin discrimination, which occurs when a person can differentiate between relatives and non-relatives and preferentially direct help towards them, is the second process for producing enough high relatedness to make kin selection essential. Several cooperative breeding vertebrate species have shown this by feeding their young more often than those of distant relatives. On the other hand, nothing is known about the significance of kin discrimination in microbes. A notable example is the development of the sex ratio in the transmission stages of malaria parasites, where there is evidence both in favour of and against the parasites determining relatedness.

Specificity is one way that kin discrimination in microbes could manifest. Production of public commodities would encourage selection to produce highly specialised molecules that other lineages could not use. This is supported by the fact that different strains of P. aeruginosa have different abilities to take up iron that has been cheated by pyoverdines generated by other strains and different forms of the pyoverdine siderophore that are formed. The genes responsible for the generation of pyo-verdine may also be being selected for novelty and specificity, according to sequencing datarestricted dispersion and kin selection. Kin selection needs cooperating people to be sufficiently closely related to one another. Hamilton proposed two potential explanations for this. Secondly, there would be a tendency for restricted dispersion to keep families together. Given that neighbours often are related, indiscriminate kindness to all neighbours will be favoured in this situation. Due to the fact that asexual reproduction results in single cells colonising and expanding in a limited region, this sort of mechanism is likely to be very significant in microbes. If r = 1, the people engaging in this scenario will be clonal, which may promote the development of cooperation.

It has been claimed that this kind of kin selection, which involves restricted dispersion, is crucial for the creation of public goods, it applies to any product for the general public that is distributed on a scale where microorganisms are often near relatives. The multiple-generation selection experiment on P. aeruginosa's production of siderophores provided experimental evidence for this theory. When cultivated under circumstances of high relatedness, a wild-type strain that generated siderophores was shown to outcompete a selfish mutant strain, but not when relatedness was lower. By enabling the bacteria to develop and communicate in groups descended from a single clone or from two clones, relatedness was altered.

Immediate Advantages of Collaboration For Fitness

We spoke about how kin selection may explain altruistic cooperation in the section above. The alternative argument for cooperation is that the person who engages in cooperative behaviour directly benefits from it in terms of fitness. Cooperation is not altruistic in this situation; rather, it is mutually advantageous. In this part, we look at two situations where there might be direct advantages to cooperation: first, when there is a common interest among people, and second, when there is a way to enforce cooperation or take away the benefit of cheating a shared interest.

When people have a common selfish interest in collaborating, selection will favour that cooperation. When an otherwise selfish behaviour helps another person, such as when one person's waste product serves as a nutrient for another, this is the most basic scenario that might result in the emergence of cooperation. Regardless of what other people do, the behaviour in this instance is mutually advantageous, offering a direct benefit to the actor who

engages in it while simultaneously benefiting another person. As shown when two interacting people each feed on a by-product of the other, benefits may flow in both ways. Both various phenotypes of the same species and distinct species may produce these byproducts [7]–[9].

DISCUSSION

Since the advantage to neighbours is only a byproduct of selfish behaviour, we would not classify these behaviours as cooperative. We contend that a behaviour should only be categorised as cooperative if it is continued, at least in part, due to the receiver benefiting from it. Cooperation, however, might develop in reaction to side advantages. For instance, if individuals A and B consume each other's byproducts, selection may favour more byproduct production by person A since it would make individual B perform better and produce more byproducts, which individual A may utilise. Individual A would be acting in a cooperative manner in this situation.

The greater honeyguide, an African bird that directs people to beehives where they consume the leftover honey from human foraging, is a well-known example of by-product reciprocity. Another example is in cooperative breeding vertebrates, where cooperative behaviour may increase group size and hence increase survival rates. In vertebrates, assisting is often focused towards family members, thus this gives a good illustration of how cooperation may result in both immediate and long-term advantages. If collaboration has synergistic advantages or if the underlying ecosystem permits cooperators to form enduring relationships, selection for cooperation is boosted.

Multi-species biofilms may experience direct advantages from cooperation if there is a net benefit, meaning that the gain from the cooperative behaviour outweighs the expense of engaging in it. Many biofilms include a variety of species. For instance, up to 0 species of bacteria may be involved in the colonisation of human teeth and the oral mucosa, with significant room for interspecies cooperation or conflict4. According to research on the early colonisers Streptococcus oralis and Actinomyces naeslundii, these two species work together to flourish where neither can by itself. Another example is the potentially fatal mixed-species biofilm between P. aeruginosa and Burkholderia cepacia that may develop in the lungs of cystic fibrosis patients [10], [11].

The issue with collaboration is that people might be chosen to pursue their own self-serving goals at the expense of the local group's overall output. Individuals can only boost their reproductive success by raising the group's production if the chances for effective cheating are eliminated. Since the plant penalises the rhizobia in a nodule by reducing the oxygen supply to that nodule, which significantly slows the bacteria's rate of development, experiments have demonstrated that cooperation is advantageous.

While evaluating the aforementioned theories of cooperation, it is important to keep in mind that the various mechanisms are not mutually exclusive. We've previously spoken about the direct and indirect advantages that actions like creating public goods may have. Another example is that by-product advantages amongst family members will result in both a direct and an indirect benefit of collaboration. Similar to this, even while collaboration is encouraged among relatives, if there is a system in place to suppress interrelational rivalry, this might result in even greater levels of cooperation [12], [13].

CONCLUSION

A crucial initial step in the study of any characteristic in evolutionary biology is to show how it affects fitness in a social setting. This entails weighing the advantages and disadvantages of

a behaviour for both the actor and the receiver. The availability of mutant strains that lack the potentially sociable characteristic often makes it easier to tackle such issues in microbes. In light of this, the first experiment compares the relative fitness of the wild type and the mutant in both single-culture and mixed environments. If this can be done in situations when the behaviour is and is not encouraged, it is very instructive. For instance, changes in the availability of iron might affect how beneficial siderophore production is. As an example, the bacteria Vibrio fischeri expensively luminesce in the light organ of their host squid; this is confirmed by the fact that mutants that cannot luminesce are unable to colonise light organs. We assume that comparable mechanisms might explain numerous occurrences of cooperation between species. Collaboration in multi-species biofilms is another option.

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CHAPTER 21

AN ANALYSIS OF BACTERIAL BIOFILMS

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ABSTRACT:

Bacterial biofilms are formed by communities that are embedded in a self-produced matrix of extracellular polymeric substances. Importantly, bacteria in biofilms exhibit a set of 'emergent properties' that differ substantially from free-living bacterial cells. Bacterial biofilms are intricate communities of microbes that stick to surfaces and are encased in an extracellular matrix that they self-replicate. Due to their significance in a number of disciplines, including engineering, medicine, and environmental research, bacterial biofilms require further investigation. By analysing recent studies on the mechanical characteristics, formation, detection, and treatment approaches of biofilms, this research paper offers an examination of bacterial biofilms. In order to facilitate accurate study and comparison of microbiological methods, the paper emphasises the significance of creating a uniform technique for the mechanical characterisation of biofilms. The alternate lifestyle that bacteria adopt during biofilm development, which helps to facilitate and/or prolong survival in various conditions, is also covered in the study.

KEYWORDS:

Bacterial Biofilms, Features, Bacterial Cells, Environmental Research.

INTRODUCTION

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. Finally, we highlight the value of an ecological perspective in the study of the emergent properties of biofilms, which enables an appreciation of the ecological success of biofilms as habitat formers and, more generally, as a bacterial lifestyle.Biofilms have been defined as 'aggregates of micro-organisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric sub- stances that are adherent to each other and/or a surface'. The term 'aggregate' accounts for the fact that most cells in multilayered biofilms experience cell-to-cell contact, either in surface-attached biofilms, in which only one layer is in direct contact with the sub-stratum, or in flocs, which are mobile biofilms that form in the absence of any substratum. Through intercellular interactions, both social and physical, together with the properties of the matrix, the biofilm lifestyle is clearly distinct from that of free-living bacterial cells. Thus, biofilm communities have emergent properties; that is, new properties that emerge in the biofilm that are not predictable from the study of free-living bacterial cells [1]–[3].

Biofilms are one of the most widely distributed and successful modes of life on Earth, and they drive bio- geochemical cycling processes of most elements in water, soil, sediment and subsurface environments. Biotechnological applications of biofilms include the filtra- tion of drinking water, the degradation of wastewater and solid waste, and biocatalysis in biotechnological processes, such as the production of bulk and fine chemicals, as well as

biofuels. All higher organisms, including humans, are colonized by microorganisms that form biofilms, which can be associated with persistent infections in plants and animals, including humans, and with the contamination of medical devices and implants. Furthermore, biofilms are responsible for biofouling and contamination of process water, deterioration of the hygienic quality of drinking water and microbially influenced corrosion. Figure 1 bacterial biofilms.

Figure 1: Bacterial Biofilms.

Biofilms are complex systems that have high cell den- sities, ranging from 8 to cells g–1 wet weight,and typically comprise many species. A further source of heterogeneity is the ability of cells in biofilms to undergo differentiation, which can be triggered by local conditions, and coordinated life cycles that include stage-specific expression of genes and proteins, as is typical for the growth and development of microorganisms in spatially heterogeneous ecosystems. The emergent properties of biofilm communities comprise 'novel structures, activi- ties, patterns and properties that arise during the process, and as a consequence, of self-organization in complex systeems' occur concomitantly and lead to biogenic habitat formation. Fundamental to these emergent properties which include the formation of physical and social interactions an enhanced rate of gene exchange and an increased tolerance to antimicrobials is the role of the self-produced EPS matrix that encases the cells of the biofilm and is mainly composed of polysaccharides, proteins, lipids and extracellular DNA.

The formation of the matrix is a dynamic process and depends on nutrient availability, the synthesis and secretion of extracellular material, shear stress, social competition and grazing by other organisms. Not surpris- ingly, the production of the matrix incurs a considerable energetic cost; however, this cost may be evolutionarily justified, owing to the structural and physicochemical centrality of the matrix to the formation and function of the biofilm, without which the beneficial emergent properties of biofilms would not arise. The matrix is an interface, or rather an 'interspace', between the biofilm and its environment that defines processes inside the biofilm and interactions with the external world. The matrix also confers a spatial organiza- tion on biofilms, from which they derive steep gradients, high biodiversity, and complex, dynamic and synergistic interactions, including cell-to-cell communication and enhanced horizontal gene transfer.

In this Review, we contend that the concept of the biofilm as an emergent form of microbial life relies on the supracellular organi- zation that arises from the formation of the EPS matrix. To support this view, we describe how the matrix pro- vides structural and functional benefits to the biofilm, such as hydration, resource capture, digestive capacity and protection from antimicrobials, in addition to facil- itating intercellular interactions that can enhance the metabolic capacity of cells in the biofilm and resistance to antimicrobials. Finally, we consider the role of eco- logical theory in understanding the social interactions that exist in biofilms, and, conversely, the potential of the study of biofilms to inform ecological theory. This ecological perspective highlights the importance of distinguishing between single-species and multispecies biofilms, and the need to study biofilms that more closely reflect the complex communities that are frequently found in nature rather than the

single-species biofilms that are most often studied in the laboratory [4]–[6]. Figure 2 biofilm infections.

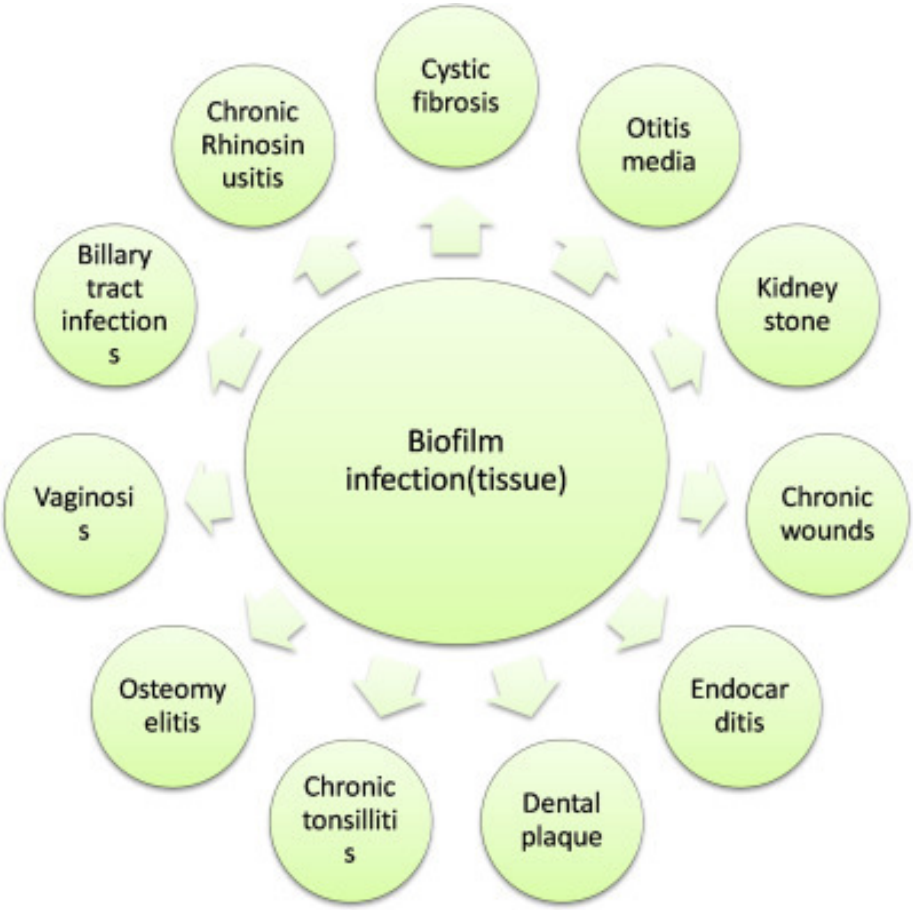

Figure 2: Biofilm infections.

The Biofilm Matrix

Most of the biomass of the biofilm comprises hydrated EPS rather than microbial cells. The self-organization of EPS molecules in the matrix is based on inter- molecular interactions between EPS components, which also determine the mechanical properties of the matrix, and the physiological activity of the organisms in the biofilm. EPS molecules mediate the formation of the biofilm architecture, which is a continuous, dynamic process that produces a spatial organization in which cells in the biofilm cluster in microcolonies. A very elegant recent study described microbial clusters in Escherichia coli biofilms with a complex supracellular architecture that is responsible for spatial physiological differentiation. Single-particle tracking of function- alized microbeads in combination with microrheology revealed that E. coli biofilms have a height-dependent charge density that changes over time. Furthermore, EPS molecules fill and shape the space between the cells of the biofilm, directly determining the environment and living conditions of the cells and providing mechan- ical stability to the biofilm. Particularly interesting is the role of eDNA; for example, the cationic exopolysac- charide Pel crosslinks eDNA in Pseudomonas aerugi- nosa biofilms, which provides structural integrity to the matrix, and DNABII binding proteins are thought to enable the formation of uropathogenic E. coli biofilms by stabilizing the secondary structure of eDNA. eDNA was also found to support the formation of a stable fil- amentous network structure in biofilms of an aquatic bacterium. The main component of the matrix is water, which contains the structural and func- tional components of the matrix: soluble, gel-forming

polysaccharides, proteins and eDNA, as well as insol- uble components such as amyloids, cellulose, fimbriae, pili and flagellae. Pores and channels between microcolonies that form voids in the matrix were recently shown to facilitate liquid transport, inspiring the concept of a 'rudimentary circulation system' for the biofilm.

In some cases, structural components of the matrix may also have other functions that benefit the biofilm. For example, in biofilms that are formed by E. coli, the main structural component of the matrix is the curli protein, which together with cellulose contributes to the desiccation tolerance of the biofilm, and Bacillus subtilis uses proteins called hydrophobins to form highly hydrophobic biofilms that float at the air–liquid interface. Other functional components of the biofilm matrix include proteinaceous filaments and nanowires that are capable of electron transport, and methods from materials science and biophysics are increasingly being used to interrogate the physical properties of bio- films, including the use of rheological, ecomechanics and electrochemical methods to investigate electrogenic properties of biofilms.Finally, in Gram-negative bacteria, enzymes that are packaged in extracellular membrane vesicles can contribute to the degradation potential of the matrix. Thus, the matrix is not simply an amorphous gel that is composed of poly- saccharides, but instead has a very heterogeneous yet highly ordered composition that includes a wide range of biopolymers that contribute to its function and emergent properties.

DISCUSSION

The non-rigid structure of the biofilm, in which distinct zones have substantially different viscosities, allows for the movement of cells in the matrix, with consequences for porosity, mechanical properties and microrheology. Common observations include the vertical migration of bacterial populations, such as in hypersaline microbial mats, and migration as a collaborative effort of pop- ulations that involves the division of labour. This is particularly well exemplified by recent observations that showed that a subpopulation of motile, plank- tonic Bacillus thuringiensis cells is able to tunnel deep into the biofilm structure at high speed. Swimmers that tunnel through the biofilm matrix create transient pores that increase mass transfer in the biofilm. Other recent observations showed that active dispersal of the biofilm can occur by partial matrix degradation, and together these observations lead to a conclusion that the highly organized biofilm matrix is not an end-point, but that instead the biofilm matrix is continuously remodelled. This remodelling is essential for the biofilm to respond to changes in the environment, such as hydrodynamic shear, or to form streamers that facilitate the colonization of a surface.

In the remodelling process, specific enzymes degrade and reconfigure the biofilm, which not only results in passive sloughing but also in active dispersal of the biofilm and subsequent surface recolonization. An important emergent property of the biofilm that is conferred by the matrix is tolerance to desiccation, as microorganisms in the environment regularly experience water stress. Indeed, bacteria in the biofilm actively respond to desiccation by the production of EPS molecules, which, owing to the high proportion of hydrated polymers in the EPS matrix, protects the bio- film from desiccation by acting as a hydrogel that retains water. Furthermore, skin formation by the uppermost EPS layers leads to an effective evaporation barrier. In a study that investigated the effect of desiccation on groundwater biofilms, the enzymatic activity of desic- cated samples was fully restored following a return to wet conditions. Thus, the biofilm mode of life is expected to provide much better protection against desiccation than that of free-living bacterial cells, which lack the benefits of the EPS matrix.

Resource capture by biofilms. The matrix enables the biofilm to capture resources such as nutrients that are present in the water phase of the biofilm or that are associ- ated with the substratum on which the biofilm is growing. Nutrient acquisition is an essential process for all organisms, and biofilms have developed a very efficient capture strategy for nutrients that exceeds that of free-living bacterial cells. The strategy relies on the passive sorption properties of the sponge-like EPS matrix, which influence the exchange of nutrients, gases and other molecules between the environment and biofilms on a global scale.Biofilms are complex sorbent systems with different sorption mechanisms and binding sites in the cytoplasms of biofilm cells, the cell walls of biofilm cells and the EPS of the matrix. These binding sites include both ani- onic and cationic exchangers, which means that a very wide range of substances can be trapped and accumulated for possible consumption by cells in the biofilm, even when such compounds are present at very low concen- trations. Figure 3 peptidomimetic polyurethanes inhibit bacterial biofilm formation and disrupt surface established biofilms.

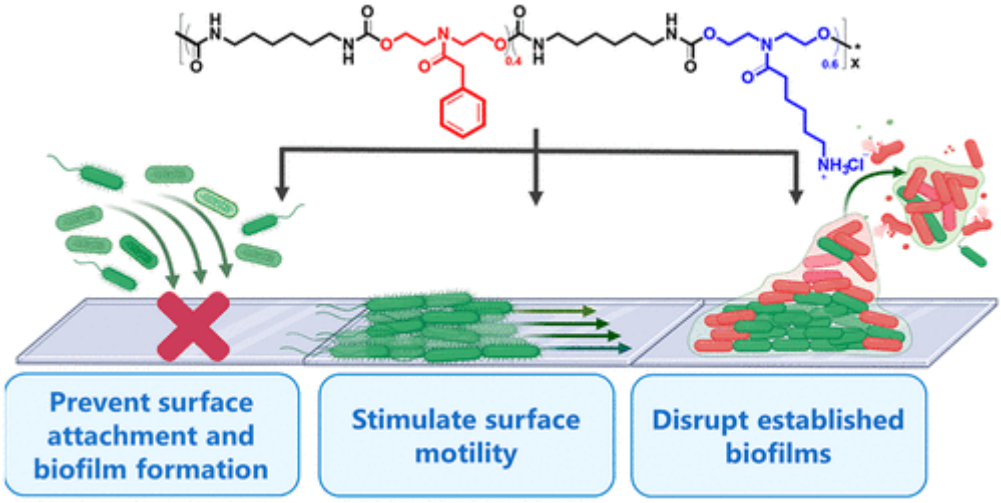

Figure 3 Peptidomimetic Polyurethanes Inhibit Bacterial Biofilm Formation and Disrupt Surface Established Biofilms [Google].

This potent sorptive capacity enables biofilms to grow even in highly oligotrophic environments. Sorption by the biofilm is not compound specific, which means that not only nutrients, but also toxic substances, can accumulate in biofilms, and compounds such as erythro- mycin, ethylsuccinate, acetaminophen, acidic pharma- ceuticals, steroidal hormones and 4-nonylphenol compounds have been found in biofilms. Surprisingly, even non-polar substances, such as benzene, toluene and xylene, can accumulate in the EPS matrix, even though it is highly hydrophilic and has no obvious lipophilic bind- ing sites. If they are not degraded, sorbed substances will be released into the water phase from the matrix if there is a concentration gradient towards water, or they will otherwise remain in the biofilm until it decomposes. Therefore, biofilms act both as a sink and a source of contaminants. Interestingly, biofilms respond dynam- ically to sorbed substances. For example, in response to exposure to toluene, biofilms of Pseudomonas putida produce EPS with a greater number of carboxyl groups, which, as anions, can lead to an increased ion exchange capacity for cations. Other anions can also be deposited in biofilms, such as the phosphate ions that enhance the mechanical stability of the highly structured, multigenus biofilms in dental plaques [7], [8].

Particularly intriguing is the dependency of antimicrobial tolerance on the biofilm lifestyle, as this phenotype is lost following dispersal3, 4. This depend- ency seems to be explained, in

part, by the concealment of cells in the matrix, which provides protection from antimicrobials, although how this protection occurs is not yet fully understood, as antimicrobial tolerance remains at concentrations of antimicrobials that are above the saturation point of diffusion–reaction inhi- bition. Slow growth states, such as dormancy, are also expected to contribute to the tolerance that is observed in biofilms; however, future studies will need to estab- lish whether dormancy is a common feature of biofilms for many organisms, rather than the small number of organisms studied in the laboratory to date, and, thus, whether biofilms are commonly a reservoir of cells in a VBNC state, possibly in starvation zones of the biofilm. Such a finding would have immense implications for the treatment of microbial infections, for the disinfection of medical devices and for an improved understanding of microbial ecology, although these applications would require further work to identify the conditions that ini- tiate dormancy or resuscitation of cells in a biofilm [9]–[11].

CONCLUSION

. An increased emphasis on biofilm community biology will also need to address the role of interkingdom interac- tions between the diverse array of microbial organisms that can be present in mixed-species biofilms. Indeed, by taking into account the structure–function contributions that can be made to interkingdom biofilms by organisms such as viruses, archaea, protozoa and fungi, studies of biofilm communities will more closely reflect the true compositions of biofilms in many natural habitats. In order to probe the biofilm properties for detection and characterization, the study further investigates the use of real-time analysis and high temporal and spatial resolution. The research also emphasises the significance of using microscopic methods to examine the matrix composition and localization of biofilms. The results of this study point to the relevance of an interdisciplinary understanding of bacterial biofilm behaviour for preventing their formation, treating infections caused by biofilms, dislodging stubborn biofilms, or taking advantage of the metabolic capabilities of biofilms.

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CHAPTER 22

EXPLORING THE ROLE OF SURFACE PROTEINS

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ABSTRACT:

Physiology and pathogenesis of bacteria are greatly influenced by surface-associated proteins, which are the main targets for vaccine development. The identification and development of vaccine targets are being greatly accelerated by recent developments in the highly accurate definition of the proteins associated with and protruding out of bacterial cells. The advent of the genomic era and high-throughput technologies marked a turning point in the search for vaccines. The nearly complete bacterial proteomes can now be scanned by in vivo and in vitro assays to identify the few protective antigens that can be included in vaccine formulations thanks to the ability to sequence whole genomes and produce hundreds of recombinant proteins quickly and efficiently. Stephen Johnston and colleagues developed the first "from-genome-to-vaccine" strategy. They used DNA immunisation and a plasmid library of the entire Mycoplasma genome to choose the genes that shield mice from Mycoplasma challenge.

KEYWORDS:

Surface Proteins, DNA,Microorganisms, Microbiology.

INTRODUCTION

Although the strategy was abandoned due to the ineffectiveness of DNA immunisation when used in high-throughput modalities, it still holds a special place in the history of vaccination because it laid the groundwork for later, more successful methods. One of these methods entails purifying each recombinant protein, screening it in the appropriate "surrogate of protection" assays, and then cloning and expressing a significant portion of the bacterial protein repertoire. Ling Lissolo and colleagues initially tried this strategy with unsure results, but Pizza et al., Maione et al., and Stranger-Jones et al. later demonstrated that it was incredibly effective for a growing number of pathogens. These vaccines are currently undergoing advanced clinical trials and will soon be available on the market. A second method is shotgun cloning of a specific pathogen's whole genome in order to produce expression libraries of Escherichia coli clones that express protein domains on their surface. The discovered immunogenic proteins are next examined for their ability to induce protective immunity in animal models after the expression libraries have been screened using sera from individuals infected by the pathogen of interest [1]–[3].

The fundamental drawback of the aforementioned methods is that in order for them to work, a large number of proteins must undergo labor- and time-intensive protection experiments that often entail vaccination and challenge of animals.The components of all antibacterial subunit-based vaccines, whether they are already on the market or in advanced stages of research, may be classified as surface-exposed compounds or secreted toxins. Hence, the creation of novel vaccines would be significantly sped up should efficient techniques capable of differentiating these compounds from the vast majority of bacterial components become accessible.

Major Developments

Currently, methods for bioinformatics can predict the surface proteins of bacteria. Yet, a significant barrier to vaccine development is that in silico studies are currently unable to provide reliable quantitative data on surface protein expression. With the purpose of identifying the proteins that are connected with bacterial surfaces, many experimental techniques have been reported. They typically rely on chemical-physical techniques to separate the various cellular compartments, gel electrophoresis or chromatography to separate membrane- and cell-wall-enriched fractions, and mass spectrometry analysis of resolved fractions for protein identification. These methods often have two serious drawbacks. Secondly, partial efficiency of fractionation techniques used to separate the membrane fraction from other cellular compartments means that many cytoplasmic proteins often contaminate the protein mixture being analysed by mass spectrometry. Second, the majority of the techniques created do not provide details on the topological arrangement of the proteins linked to or included in the bacterial membrane. Given that the protein domains sticking out of the surface are those engaged in the interaction with the host immune system, this knowledge is very pertinent for vaccination applications [4]–[6].

Selective biotinylation of entire bacteria with various biotinylating agents and subsequent mass spectrometry analysis of labelled proteins are other techniques for characterising surface proteins. These techniques, although highly instructive, also have the drawbacks of being inadequately quantitative and selective.Lately, two methods that at least partly get around the aforementioned restrictions have been offered. The first of these methods is intended to identify surface-exposed proteins in Gram-positive bacteria. It entails the proteolytic enzymes "shaving" the bacterial surface in a manner that protects the health and viability of the bacterial cells. After digestion, the liberated peptides are isolated from the "shaved" bacteria and put under the microscope for protein identification using mass spectrometry. The method has been successfully used to the detection of group A and group B Streptococcus surface proteins. The majority of the time, fluoresence-activated cell sorting study has shown that antibodies against the discovered proteins' recombinant versions bind live bacteria, hence confirming the proteins' presence on the bacterial surface. One important feature of this strategy is that there is very little intracellular protein contamination; as a result, the proteins detected by mass spectrometry are genuine surface-exposed proteins. The proteolytic peptides produced by the enzymatic treatment also correspond to the exposed portion of the proteins since only the projecting domains are accessible to proteases; their characterisation therefore offers helpful information on the topological organisation of each detected protein.

Due to the relative fragility of the cells, which often perish during protease treatment, the "shaving" method is challenging to utilise with Gram-negative bacteria. A new strategy has recently been devised for these bacteria that takes use of their inherent inclination to release outer membrane vesicles. The number of released OMVs, which is often too little for practical use, may significantly rise and reach values of several milli- grammes per litre of culture if certain mutations are chosen. OMVs are quite tiny, making it simple to remove them from bacterial cells using centrifugation or ultrafiltration before analysing them using mass spectrometry to identify the proteins.

Recent reports describe the proteome characterisation of OMVs from a pathogenic E. coli strain and a group B isolate of Neisseria meningitidis. As predicted given that OMVs are created by the outer membrane "budding out," the findings demonstrate that OMVs nearly exclusively comprise outer membrane proteins and very few periplasmic proteins. Preliminary findings, however, show that OMVs may be shaved with proteases without

losing their integrity, unlike parent cells, permitting topological investigations of surface exposed proteins.

Accurate maps of bacterial surfaces that describe protein organisation in both topological and quantitative dimensions will soon be available thanks to these newly discovered technologies. According to the data currently collected, there are just a few tens of exposed membrane proteins that are produced at a level high enough to be the focus of protective immune responses. The majority, if not all, future vaccines may be discovered among them, as experimental evidence has shown.

Upcoming Directions

In surface proteome analysis, there are two key issues that need to be resolved since they might have significant implications for the creation of new vaccines. First, it is widely known that bacteria modify the arrangement of their surface proteins in response to environmental factors. Surface proteomes have only ever been studied up to this point under very precise, often artificial settings. Determining how proteins arise and vanish on the bacterial surface would thus provide light on the physiology and pathogenesis of bacteria. Second, it is anticipated that certain proteins with accessible surfaces would interact to form useful protein complexes. The formation of protein complexes on the bacterial surface is still mostly unknown, and further research is needed in this area [7]–[9].

Adhesion and Host Cell Invasion

To colonise and establish infection, all bacterial pathogens must get through a variety of anatomical, physiological, and immune barriers in addition to getting nutrients. An essential technique for bacterial colonisation, immunological evasion, and spread is adhesion to and penetration of host cells. To mediate binding and absorption by various cell types, gram-positive bacteria use a variety of methods. In order to permit binding to tissue or cells that contain polymorphism ligands or receptors in a different host species, such interactions may need diversification or may favour establishment in a specific niche prevalent across several host species.

The Listeria monocytogenes internalin A

The internalin is a well-known instance of a CWA protein mediating both niche- and host-specialized cellular internalisation. a protein from the very aggressive foodborne bacterium L. monocytogenes, which also causes encephalitis in ruminants, meningitis in infants, and miscarriage, stillbirth, and early labour in pregnant women. InlA promotes niche-specialization by interacting with the N-terminal portion of E-cadherin produced by epithelial cells, including those found on the placental villous trophoblast barrier. This association primarily enables L. monocytogenes to transcytose on the luminal surface of intestinal goblet cells or on intercellular junctions that occur normally during gut homeostasis in order to pass past the intestinal epithelial barrier where E-cadherin is exposed. It has been shown that natural premature stop codons in the inlA gene of L. monocytogenes clinical isolates are associated with a reduced capacity to invade intestinal epithelium and an increased infective dose in experimental models. Heterologous expression of InlA confers the capacity for internalisation to the invasion-deficient Listeria innocua.

Stronger binding to human E-cadherin was discovered compared to murine E-cadherin, which provided evidence for host-specialization in the crystal structure analysis of the InlA interaction with E-cadherin. Elegant mutational studies showed that changing host-tropism just required one proline residue at location of E-cadherin. Strong binding is facilitated by the

presence of Pro in E-cadherin, which normally exists in humans, rabbits, and guinea pigs, while Glu, which naturally occurs in rodents, results in a lower affinity. Of note, cows also encode Pro in bovine E-cadherin, and rabbits are thought to be L. monocytogenes' natural hosts. As a result, InlA of L. monocytogenes has evolved to enhance niche- and host-specific invasion of intestinal and placental epithelial cells in the native rabbit host, whereas conserved residues in human and bovine E-cadherin support the ability for zoonotic infection.

Adhesion Molecule from The Streptococcus Uberis

The main culprit of bovine mastitis, Streptococcus uberis, has likewise developed an invasion and adhesion mechanism that is host- and niche-specific. When a cow has mastitis, lactoferrin, an antibacterial glycoprotein that sequesters iron, is strongly produced in the milk. Several bacterial pathogens have developed strategies for interacting with lactoferrin because of its prevalence and significance in host defence. Two lactoferrin-binding proteins that are encoded by S. uberis show preference for bovine lactoferrin over the human version and encourage evasion of lactoferrin's antibacterial activities in the mammary gland niche. To increase attachment to and internalisation of S. uberis into bovine mammary epithelial cells, the S. uberis adhesion molecule specifically employs lactoferrin as a cross-bridge with host interlectin-1. A critical function for SUAM in the pathogenesis and niche-specificity of S. uberis has been shown by the attenuation of virulence in an experimentally infected mammary gland by a mutant S. uberis strain defective in SUAM expression.

Immune Evasion Innate

Gram-positive bacteria's ability to cause disease depends on their ability to overcome the host immune system in addition to acquiring vital nutrients and invading host cells. Numerous innate immune mechanisms, such as the formation of blood clots at wound sites and inflamed blood vessels and the activation of the complement cascade that results in intracellular killing by phagocytic cells like neutrophils, macrophages, and dendritic cells, have evolved to protect against bacterial infections. In response, Gram-positive bacteria may use surface proteins to engage with host proteins like plasma glycoprotein fibrinogen to block both hemostasis and phagocytosis and complement factors like factor H and CD to prevent complement deposition in a niche- or host-specific way.

Fibrinogen Interactions with Staphylococci

Fibrinogen, which is essential for hemostasis, is composed of - and -chains in the form of dimers of trimers. Because of fibrinogen's widespread use and availability, a variety of bacterial pathogens have developed strategies to manipulate it for adhesion to host cells, abscess development, and immune evasion. A universal yet host-specific interaction between Staphylococcal species, such as S. aureus, S. lugdunensis, and S. pseudintermedius, and the C-terminal of the fibrinogen -chain prevents fibrinogen-mediated coagulation and platelet aggregation. Due to a single amino acid substitution in the ovine fibrinogen -chain, the fibrinogen -chain binding for clumping factor A of S. aureus exhibits reduced binding to bovine fibrinogen and no detectable ovine fibrinogen-binding. However, the fibrinogen -chain binding is equivalent for human, canine, feline, murine, and porcine fibrinogen. In mouse infection models, this host-specific fibrinogen-interaction of ClfA is critical for S. aureus-mediated sepsis as well as facilitating bacterial aggregation and phagocytosis suppression, two important innate immune evasion tactics.

The repetition portion of the fibrinogen -chain, which demonstrates both inter- and intraspecies polymorphism, interacts with other Staphylococcal CWA proteins in a more host-specific manner. S. pseudintermedius surface protein L exhibits canine-specific high-

affinity binding to the repeat region of the canine fibrinogen -chain, whereas S. aureus clumping factor B interacts exclusively with repeat 5 of the human fibrinogen -chain, enabling human-specialized platelet aggregation. SpsL is exceptional in that it exhibits both a secondary, weaker binding relationship that is also seen with human fibrinogen and canine-specific binding to the repetition region of the fibrinogen -chain. This canine-specific fibrinogen interaction encourages S. pseudointermedius aggregation and neutrophil phagocytosis inhibition, illustrative of the function of fibrinogen-binding as a host-specific immune evasion tactic. The Srr1 and Srr2 glycoproteins of Streptococcus agalactiae specifically bind to human repeats 6-8 when adhering to the fibrinogen -chain, suggesting that this region of fibrinogen is the focus of host-specialization for a variety of Gram-positive bacteria.

Streptococcus Pyogenes' M Protein

Streptococcus pyogenes is a significant human-specific pathogen that uses a variety of innate immune evasion tactics to produce severe invasive infections including necrotizing fasciitis as well as acute pharyngitis and impetigo. The M protein, which is the most prevalent CWA protein on the surface of S. pyogenes, is related with certain habitats like the pharynx or skin. The M protein possesses a variety of host ligands that support immune evasion as well as host cell adhesion. Complement inhibition is caused by the binding of M protein to complement elements such factor H, C4BP, and CD. M protein has been shown to interact in a human-specific way with CD complement regulatory protein, which facilitates the cleavage of complement components C3b and C4b on host cells. The binding to keratinocytes and invasion of lung epithelial cells caused by the interaction of the M protein with human cellular CD eventually results in cell death. Since soluble CD is lost from the host cell during epithelial cell death, less S. pyogenes is killed in whole blood as a result of its interaction with M protein. The higher whole blood survival in transgenic mice that express human CD serves as proof that this immune evasion mechanism is exclusive to humans. Furthermore, more prone to experimental infection, these human-expressing CD mice developed necrotizing fasciitis following subcutaneous injection, as well as higher mortality and bacterial levels after a bloodstream infection. As a result, S. pyogenes has created a complement inhibition-based immune evasion technique that is exclusive to humans.

Equi's Immunoglobulin Binding

The fibrinogen-binding protein of the equine-specific S. equi subsp. equi, the cause of the respiratory illness strangles in horses, provides a last illustration of host-specific immune evasion. FgBP, a CWA protein, was first identified as a protective antigen in a mouse infection model and as a fibrinogen-binding protein with a predilection for horse Fg. It was subsequently shown that this protein did not bind to the IgG of mice, rats, goats, sheep, cows, or chickens, only to the IgG of horses, humans, rabbits, porcines, and felines. A host-specific immune evasion tactic used by S. equi subsp. equi, FgBP interacts with the Fc interdomain region of IgG4 and IgG7 to impair complement deposition and antibody-mediated activation of the classical complement pathway, increasing survival in horse whole blood.

Models for the Analysis of Host- and Niche-Specific Cell Surface Proteins

The examples given here include structural, biochemical, and molecular proof of interactions that are host- and niche-specific. Nonetheless, it may be difficult to prove the significance of these host-pathogen interactions in the right infection models, particularly when the bacteria show a human tropism. In other circumstances, an efficient model may not exist, and using big animals for experimental infections might have ethical and/or financial repercussions. It's crucial to note that small animal models may not share ligands or receptors with the native

host and would, as a result, not support the function of the questioned bacterial surface protein. In vitro host-pathogen interactions may be studied using knockdown and heterologous expression methods in cell lines, but they are unable to address the importance of the interaction in a complicated infection situation in vivo. A very interesting new field of study is the creation of three-dimensional organoid systems grown from stem cells for the study of infection biology. These systems will provide more sophisticated multicellular systems for studying host-pathogen interactions in vitro. In one such system, a 3D mammary organoid made of mouse mammary gland tissue was created and may be used to regulate lactation or involution. It is possible to create a bovine mammary organoid using a similar technique, enabling for in vitro testing of the lactoferrin-dependent internalisation of S. uberis [10], [11].

DISCUSSION

Another option is to create transgenic mice that express the receptors necessary for surface protein activity. This method was used to investigate the involvement of L. monocytogenes InlA during colonisation of the gut, in addition to the instances previously given for S. aureus IsdB and S. pyogenes M protein. Animals were genetically modified to express either a "humanised" GluPro form of the mouse E-cadherin in all E-cadherin-expressing cells or human E-cadherin from a specific promoter in enterocytes. Both methods showed that InlA is necessary for L. monocytogenes to pass the intestinal barrier, and the chimeric mice showed that both InlA and Internalin B are necessary for placental invasion during murine pregnancy. In the opposite situation, the L. monocytogenes cell surface protein InlA has undergone two single amino acid changes to become "murinized," which increased the protein's ability to attach to murine E-cadherin in vitro and caused infection of the mouse intestinal epithelium in vivo. As a result, the liver and spleen had larger bacterial burdens than the other organs, highlighting the significance of this InlA-dependent mechanism of bacterial spread. The CBL/6J mouse cell line possesses intrinsic resistance to oral challenge by Listeria, but more susceptible murine cell lines display quicker spread of the mutant strain as well as higher cytokine production, according to further usage of this "murinized" L. monocytogenes strain. Transgenesis of rats has become a reasonably simple process because to improvements in genome editing techniques, like as CRISPR-Cas systems. This has made it possible to create novel transgenic models that may be used to study the significance of host-specialized bacterial interactions in vivo. To test the activity of the S. aureus toxin LukAB in vivo, recent investigations used CRISPR-Cas altered mice expressing the human integrin component CDb.

Schematic overview of studies on the relationship between human E-cadherin and L. monocytogenes internalin A. The "murinization" of InlA with improved binding to murine E-cadherin was made possible by the crystal structure creation of InlA in complex with human E-cadherin, PDB 1O6S. There are two transgenic mice models that either express the human E-cadherin protein throughout the whole animal or just in its intestines. All three methods showed that InlA played a part in breaking through the intestinal epithelial barrier during an experimental infection with L. monocytogenes [12], [13].

CONCLUSION

Gram-positive bacteria's surface proteins are necessary for a variety of host-pathogen interactions. As a result, the acquisition of novel proteins or the diversification of existing proteins to permit binding to polymorphic ligands or receptors is often required for bacterial adaptation to a new host species or anatomical niche. Important strides have been made in our knowledge of the molecular foundation for such interactions in vitro, as shown by the

examples given in the present review. The creation of novel organoid or whole animal models of infection that permit the analysis of the function of niche- and/or host-specialized surface proteins, many of which have multiple binding tropisms involving a variety of receptor ligands, also holds great promise thanks to new techniques for transgenesis and mutagenesis. The creation of suitable experimental models will enhance the present model systems used for the assessment of innovative treatment and vaccination candidates as well as enable the validation of surface proteins that play significant roles in vivo. The discovery of new methods for infection management is crucial, especially for bacteria with high levels of antibiotic resistance that are becoming harder and harder to treat in a clinical setting.

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CHAPTER 23

MICROBIAL SECONDARY METABOLITES AND BIOTECHNOLOGY

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ABSTRACT:

The microbial world is immeasurably richer and more fascinating than we could have anticipated, as recent scientific studies has shown. Microorganisms manufacture new compounds every day, and their amazing variety has not yet fully conveyed all of their meanings. The current research challenge is to identify, from the large pool of characterised microorganisms and compounds, those that might quickly answer important questions about the health of people or animals, or more generally about the needs of society in the areas of medicine, pharmacology, nutrition, or everyday well-being. Secondary metabolites produced by microorganisms, also known as specialised metabolites, often have peculiar structures and have a significant impact on societal economics, nutrition, and health. These substances are often made up of low-molecular-mass byproducts of secondary metabolism, which occurs independently of the primary microbial development phase. These consist of substances with hormonal activity or specific effects on the metabolism of fats or carbohydrates, as well as antibiotics, pigments, poisons, enzyme inhibitors, immunomodulators, effectors of ecological competition or symbiosis.

KEYWORDS:

Secondary Metabolites, Biotechnology,Microorganisms, Compounds, Primary Microbial Development.

INTRODUCTION

As antimicrobials, antivirals, antioxidants, antitumorals, vaso-relaxants or contractants, diuretics or laxatives, several have previously made themselves known. Others are utilised as insecticides, growth stimulants, or colourants for animals or plants. Around% of the natural product-based medicines that the FDA has authorised come from microorganisms [1]–[3].Nutrient supplies, growth circumstances, feedback regulation, enzyme induction or inactivation, and other factors all fine-tune the production of specialised metabolites. Certain low molecular mass substances, as well as transfer RNA, factors, and gene products produced during post-exponential development, often affect how they are regulated. Recent studies have shown that chromosomal DNA, not plasmidic DNA, primarily codes for the generation of specific metabolites. Academic scholars studying enzymology, regulation, and differentiation now have a new theoretical area to explore since the linked pathways are still not completely understood.

New options for the characterisation and enhancement of strains of industrial microbes are now available thanks to the use of omics sciences including genomics, transcriptomics, and metabolomics. The creation of new chemicals for the pharmaceutical, nutraceutical, dyeing, or agricultural sectors is thus quite promising.Secondary metabolites generated by bacteria, actinomycetes, fungi, or microalgae are examined in this special issue under several angles

related to bioactivity or other intriguing features. A variety of scientific disciplines that bridge fundamental and practical research are combined in biotechnology. This is the first but essential step in resolving technical issues, predicting in du

It has been discovered that sponges and the bacteria that live inside of them create a vast range of bioactive secondary metabolites. Many natural substances derived from marine species have previously shown promise in the pharmaceutical industry as anti-aging and/or life-extension agents. However considerably less research has been done on the biodiversity from the southwest of the Indian Ocean, particularly with reference to anti-aging techniques. The microbial diversity of the marine sponge Scopalina hapalia was examined by metagenomic analysis in the work reported by Sad-Hassane et al. The sponge had 26 bacterial and 2 archaeal phyla, with the Proteobacteria phylum being the most prevalent. For the purpose of producing secondary metabolites, thirty isolates belonging to the genera Bacillus, Micromonospora, Rhodoccocus, Salinispora, Aspergillus, Chaetomium, and Nigrospora were grown. Certain microbial cultures' crude extracts were discovered to have activity against the following enzymes: elastase, tyrosinase, catalase, sirtuin 1, CDK7, Fyn kinase, and proteasome. These findings demonstrate the capability of microorganisms linked to an Indian Ocean marine sponge to create anti-aging substances [4]–[6].

Very diversified and common marine epiphytic bacteria must constantly fend off pressures from physicochemical factors, biotic competition, and predation in marine biotopes in order to exist. To combat hardship, these marine species have evolved defensive mechanisms, such as creating harmful bioactive chemicals. These bacteria have been shown to produce a number of active metabolites as a result of the expression of certain genes. The potential of marine epiphytic bacteria as a new platform for the development of innovative anti-nematode drugs is the main topic of Salikin et alreview. New techniques are emphasised, such as culture-independent high-throughput strain discovery.The review by Nawaz et al. focuses on pigments and gives an outline of the processes involved in marine bacteria producing bio-pigments and their usage. It draws attention to a variety of compounds that are already highly prized in the industry, including prodigiosin, astaxanthin, violacein, zeaxanthin, lutein, and lycopene.

Since they may create a variety of secondary metabolites, bacteria that are linked with insects are thought to have a role in the life cycle of their hosts. Undoubtedly, one of the less-explored sources of novel active chemicals is these strains. The diversity of a strain's culture circumstances may cause the activation of several biosynthetic gene clusters with the purpose of effectively identifying novel bioactive compounds. This OSMAC strategy is based on the idea that certain microbial metabolites may not be generated under a particular set of physicochemical circumstances but may do so if those conditions are altered. The Streptomyces sp. GG strain of actinobacteria, which was isolated from the stomach of Tenebrio molitor, showed a considerable improvement in the generation of the cyclic pentapeptides pentaminomycins C, D, and E as a result of these two methods. The non-ribosomal peptide synthetase gene cluster analysis suggested that macrocyclization can be catalysed non-canonically by penicillin binding protein -type TE and that the uniqueness of the compounds, based on structural variations, originates from the low specificity of the adenylation domain in the non-ribosomal peptide synthetase module. Pentaminomycins C and D also shown notable autophagy-inducing activity and provided in vitro cytoprotection against oxidative stress generated by menadione.

The investigation of secondary metabolites derived from microbial co-cultures using metabolome technologies is one novel area of research. Three actinomycetes strains were identified as new species of the genera Micromonospora, Nocardia, and Gordonia among the

diverse microbiota recovered from the Red Sea sponge Coscinoderma mathewsi. This study by Shamikh et al. showed that Micromonospora sp. UA could produce metabolites like a chlorocardicin, neocopiamycin A, and chicamycin B that were not present in the corresponding monocultures when it was co-cultured with two mycolic acid-containing actinomycetes, Gordonia sp. UA and Nocardia sp. UA. This suggests that mycolic acid has an impact on the stimulation of cryptic natural product biosynthetic pathways and shows that, under non-traditional culture settings, silent biosynthetic gene clusters may demonstrate their exceptional capacity for the creation of secondary metabolites.

Serratia marcescens, an opportunistic pathogen, produces siderophores, such as serratiochelins, which are specialised substances with a strong affinity for ferric iron. Pharmaceutical companies are interested in siderophores because they can be used to treat iron overload illnesses or make siderophore-antibiotic drug conjugates that help bacteria accept antibiotics uptake more efficiently. In Schneider's research, Serratia sp. and Shewanella sp. were co-cultured in an iron-depleted environment, and the uncommon siderophore serratiochelin A was isolated with high yields. Although this molecule was not found in Shewanella or Serratia axenic cultures, co-culturing may have caused the formation of the chemical, potentially as a result of the two strains' battle for iron in the culture medium. Tests showed that the antibacterial action of serratiochelin A was exclusive to S. aureus. Moreover, the chemical had harmful effects on the eukaryotic cell lines MRC5 and A.

Novel Chemicals

Streptomyces is a well-researched species that has traditionally been recognised for producing bioactive substances. Yet, strains with atypical ancestry may provide novel natural goods. For instance, the Streptomyces sp. strain IB-2A from the endemic Benedictia baicalensis mollusc from Lake Baikal synthesises three novel angucyclines as well as significant amounts of rabelomycin and 5-hydroxy-rabelomycin. Several amounts of anticancer activity were seen in the baikomycins A through C. Both rabelomycin and its 5-hydroxy derivative exhibited antiproliferative properties. In S. albus J., the gene cluster responsible for the production of baikalomycins was discovered, cloned, and expressed. The glycosyltransferase functions thought to be involved in the production of these original chemicals were able to be identified by heterologous expression and deletion studies.

The endophytic fungus Curvularia papendorfii, which was isolated from the Sudanese medicinal plant Vernonia amygdalina, produced a crude extract that had significant antiviral activity against the human coronavirus HCoV 9E and the feline coronavirus FCV F9. Furthermore, noted were an intriguing antiproliferative competence against the human breast cancer MCF7 cell line as well as a selective antibacterial activity against Staphylococcus sp. From this extract, 22 metabolites were found, and two main pure substances, including a brand-new polyhydroxyacid called kheiric acid, were described. When used against methicillin-resistant Staphylococcus aureus, kheiric acid shown excellent inhibitory capabilities. As a result, endophytes need further research since they contain a wealth of novel bioactive substances.

Growing Understanding of Bioactive Properties

Considering the social ramifications, research teams put a lot of effort into finding bioactive characteristics in bio-based compounds.To produce docosahexaenoic acid, the marine microalga Aurantiochytrium sp. is thought to be a suitable source. DHA is an essential n-3 long-chain polyunsaturated fatty acid that is important for cellular functions that support good health. UV mutagenesis was used in the work by Liu et al. to create a competitive Aurantiochytrium sp. strain with increased DHA synthesis. Comparing the transcriptomes of

the mutant and parent strains allowed researchers to identify the main genes responsible for the rising DHA buildup. The enhanced intercellular generation of DHA is correlated with the mRNA expression levels of the CoAT, AT, ER, DH, and MT genes, and these levels may be changed to regulate DHA yields in Aurantiochytrium sp. Hence, the enormous potential of microbial genetic enhancement is only starting.

Exopolysaccharide-based biofilms, which shield bacteria from harmful environmental factors, are more likely to contaminate surfaces such as those used for food or medical supplies. Using natural anti-microbial chemicals is a traditional strategy for getting rid of biofilms. To suppress the pathogenic yeast Candida albicans, Argüelles et al. devised novel chemical synergies, such as the combination of carnosic acid and propolis. Lytic phages are also used in recent advancements in biofilm removal. The lytic activity of Xcc1 was assessed in Papaianni et alinvestigation.'s in conjunction with 6-pentyl-p-pyrone linked to hydroxyapatite. By modifying the genes involved in biofilm formation and stability, the findings showed that Xcc, alone or in combination with 6PP and HA, disrupts the gene pathways involved in the creation of biofilm. This strategy may be used to combat different bacteria that produce biofilms.

Hamed et al. concentrated on endophytic fungi isolated from marine species, gathered from salty settings, with the premise that the application of antimicrobial fabrics may considerably lower the incidence of nosocomial infections. With regard to a panel of harmful bacteria and fungi, the antibacterial and antioxidant properties of fungal isolates were investigated. Initially, the strains' crude ethyl acetate extracts had antibacterial or antioxidant properties. By grafting MCT-CD onto the surface of cellulosic textiles, core-shaped hydrophobic voids were innovatively created. It was also possible to include the three most potent fungal extracts into the hydrophobic cavities. The results showed that Aspergillus calidoustus strain M3 had the second-best UV protection and the most potential improvement in the antibacterial functioning of this innovative generation of textiles. The toxicity against healthy human skin fibroblasts was found to be modest to weak. The initial stage in determining the economic viability is the large-scale manufacturing of these bioactive extracts as well as the industrial use of the technique to create eco-friendly multifunctional fabrics.

The Sayed team focused on the protein Mpro using in silico methods that have lately received interest in drug development programmes. The two replicase proteins, pp1a and pp1ab, are guaranteed to be cleaved by this crucial protease of the severe acute respiratory syndrome coronavirus 2. Virtual screens using hyphenated pharmacophoric and structural criteria were conducted using Mpro. By adapting to the catalytic site of Mpro, the Natural Products Atlas, a database of more than 0 microbiological natural chemicals, was searched for analogues demonstrating antiviral activity. Lipinski's criteria were used to identify candidates that resembled drugs. Top-scoring hits were further weeded out based on their capacity to exhibit adequate binding affinities towards the conformers of the enzyme determined from the molecular dynamic simulation. As a result, six substances with strong promise as anti-SARS-CoV-2 drug candidates were found. A potential first step towards the quick development of drug candidates against SARS-CoV-2 is further in vitro testing of the chosen compounds. 5. Difficulties in Manufacturing Processes

We can now improve our understanding of the biological processes that underlie biotechnologies thanks to omics sciences.Research on the yeast Saccharomyces cerevisiae has significantly advanced the wine business. A proteomic map can be created using Gonzáles-Jiménez et alresearch.'s to identify the protein content of sparkling wines made by various strains of Saccharomyces cerevisiae. The study demonstrates that, with the exception of the proteins Adh1p, Fba1p, Tdh1p, Tdh2p, Tdh3p, and Pgk1p, the proteins in yeasts that

are responsible for the production of the volatile compounds released during sparkling wine elaboration are quite similar in a flor yeast and a conventional one. Compared to the traditional strain, the flor yeast has increased levels of these proteins. The differing organoleptic qualities achieved while ageing with flor yeasts may be explained by the greater concentration of these proteins. The particular volatilome is affected by processes that include the released mannoproteins, autolyzed chemicals, enzymes, and certain aroma precursors. Hence, proteomic analysis may be required in order to characterise a wine's unique quality with more accuracy.

The extraordinary capabilities that genetic changes provide now allow research to encourage the creation of molecules of interest in hosts other than the molecules' original creators. It is now feasible to break free from the geoclimatic and geographical limitations that are inherent in plant cultures by using bacteria as productive hosts. Moreover, the molecules may be synthesised in inexpensive medium at quite fast growth rates, enabling the use of sunlight as an energy source for environmentally friendly growing and manufacturing procedures. The heterologous host Rhodobacter capsulatus was used by Hilgers et al. to enhance the plant sesquiterpenoid pathway leading to -caryophyllene. The highest output, 9 mgL1, was attained. The bioactivity of -caryophyllene and its oxygenated derivative was assessed against a variety of phytopathogenic fungi since sesquiterpenes often exhibit advantageous anti-phytopathogenic properties. Sclerotinia sclerotiorum and Fusarium oxysporum, two phytopathogenic organisms, were greatly slowed down by the molecules, while other organisms, such as bacteria that aid in plant development, were unaffected. As a result, the synthesis of -caryophyllene and -caryophyllene oxide in Rhodobacter capsulatus may be seen as a potential approach for the creation of natural chemicals for the control of various plant pathogenic fungi in the production of agricultural crops.

Using Corynebacterium glutamicum as the main production host, it has already been successfully shown that amino acids may be produced in a safe manner for the food and feed industries. The objective of Walter ET alresearch.'s was to prefer a particular route for the synthesis of an N-functionalized amino acid called N-methylanthranilate. This amino acid serves as a primary building block for bioactive substances including acridone alkaloids, which fight cancer, and other peptide-based medications. Unfortunately, because of poor yields or low profitability, it is difficult to effectively leverage the present methods of chemical or biological synthesis of N-alkylated amino acids. By metabolically engineering the genome-reduced chassis strain C. glutamicum C1 and introducing the SAM-dependent ANMT gene from Ruta graveloens, the study establishes a fermentative NMA manufacturing method. This modified strain produced NMA at a level never previously achieved, with a final titer of 0.5 gL-1 and a yield of 4.8 mgg1 glucose. Hence, very customised changes suited to market requirements are feasible.

The extraction stage is a crucial step in the direction of industrial production after strain characterisation or improvement and metabolite synthesis. The extraction of a microbiological product often uses solvents, which is against the idea of a sustainable environment. The azaphilone red pigments and ergosterol derivatives generated by a wild type of marine-derived Talaromyces species are reported in the research by Lebeau et al. A new method of extracting the fungal pigments has been devised that combines high pressure with environmentally safe solvents. Due to their use in the creation of fresh medicinal goods, these fungal pigments could be of interest.Yet in order to maximise the extraction of the molecules, Heroic labour must be done. Promoting the synthesis and use of microbial natural compounds while increasing the number of contaminants released into ecosystems would be counterproductive.

Developing Biotechnology and Expanding into Strange Areas

One of the pillars of the move from pilot-scale fermenters to industrial applications is improving biotechnological processes.Moreover, owing to their non-toxic, non-immunogenic, thermo-resistance, and wide bactericidal activity features, lactic acid bacteria bacteriocins are regarded as effective bio-preservative agents. It may be possible to establish effective methods for food biopreservation via the large-scale manufacturing of BLIS. The research looked at variables such the size and age of the inoculum, the initial pH of the culture medium, and the kind of nitrogen and carbon sources. This effort helped identify the ideal set of criteria based on the creation of BLIS. Conclusion: By changing the bacterium's growth conditions, bacteriocin production may be noticeably enhanced. The outcomes may then be used in larger-scale process design and optimization.

They created a 2 L stirred-tank bioreactor with an internal column utilising cation-exchange adsorption resin after showing that the buildup of by-product ammonium in the culture medium reduced gdhA P. multocida B:2 growth. By gradually diminishing the in situ inhibitory impact of ammonium, they were able to show a considerable increase in the growth performance and viability of the bacteria.It is undoubtedly necessary to apply computer and statistical methods in order to effectively increase the yields of certain metabolites.

In order to optimise the physico-chemical parameters for the synthesis of pigments and biomass in submerged fermentation by a marine-derived strain of Talaromyces albobiverticillius, Venkatachalam et al. completely use computational tools. A Box-Behnken experimental design based on a three-interlocking factorial matrix was used to accomplish the optimization, significantly lowering the number of tests needed. A response surface modelling procedure was used to anticipate the ideal circumstances after statistical analysis of the findings. This technique made it simpler to take into account the multifactorial interactions between a variety of different characteristics and made it possible to choose the ideal culture conditions quickly in relation to the many goals that were defined. As a result, the predicted model was confirmed, and the following circumstances were found to be ideal for the highest production of pigments and biomass: beginning pH of 6.4, temperature of °C, and 4 rpm of agitation for a 9-hour fermentation. This strategy makes it easier and quicker to choose the ideal experimental setups for the generation of desired metabolites.

Sugarcane distillery wasted wash is one of the most harmful industrial effluents in the realm of environment depuration. Its high mineral content, acidic pH, and chemical oxygen requirement are all contributing factors to significant environmental disturbances. The European regulation /CE encourages the creation of high added-value products from waste, which is a cost-effective method of bioremediation. Chuppa-Tostain et al. chose yeast and filamentous fungal strains to assess their potential for lowering the organic content of vinasses by seeing how they affected the parameters of COD, pH, minerals, and OD5nm. The strains examined produced the greatest results for the depollution of DSW when Aspergillus and Trametes species were used. In the next years, there will undoubtedly be a focus on research in the area of biological purification due to the rise in soil and water contamination.

Environmental contaminants include the industrial effluents from agro-food processing that are produced during the roasting of maize. Several research projects focus on the use of this wastewater as a starting point for the synthesis of bioactive metabolites by microorganisms. The study by Bacame-Valenzuela et al. focused on the culture conditions for Pseudomonas aeruginosa's generation of pyocyanin. The parameters were first tuned in a specified medium using statistical design and RSM. The improved parameters were then used in a culture using

an effluent made from alkalinized maize. Up to 3. gmL more pyocyanin was produced than in the original specified medium. In this manner, the creation of a value-added substance served as evidence of the valorization of lime-cooked maize effluent employed as a substrate for microbial growth.

In addition, Zhang et al. concentrated on the method for making lactic acid from maize stover, a typical agricultural byproduct. One of the most plentiful sources of renewable fuel is lignocellulose residue. They chose a Pediococcus acidilactici strain that is safe and has high xylose consumption, excellent lignocellulose sugar fermentation, and high temperature tolerance. They created a fed-batch simultaneous saccharification and fermentation process in a 5 L bioreactor utilising maize stover and corn steep powder as carbon sources to increase the generation of lactic acid. In relation to the NaOH-pretreatment used on maize stover and the inclusion of cellulases, the culture generated up to 4. g L-1 of lactic acid and a yield up to 0. g G-1. In addition to providing a viable candidate strain for high-titer and -yield lignocellulose-derived lactic acid synthesis, this research devised a workable and effective fed-batch technique for lactic acid manufacture from maize stover.

DISCUSSION

The future is full of new chances to advance the use of microbes as managed cell factories that produce very valuable metabolites for our daily requirements as a result of technological and scientific advancement [7]–[9].For instance, new strategies are being developed even though filamentous actinobacteria have previously been recognised and employed as sources of antibiotics. An age-old method of genome recombination called protoplast fusion greatly boosts the genetic diversity of microbes and encourages the expression of genes that are quiet or poorly expressed. The procedure requires several fusion and regeneration steps, however. Since protoplasts are required to repair the cell wall, cells must undergo recombination within a short period of time, and recombinants are often unstable. These drawbacks clearly place restrictions on the creation and use of important original metabolites. The potential of newly created wall-deficient bacteria that can grow without a cell wall is highlighted in the paper by Shitut et al. L-forms, as opposed to protoplasts, have the capacity to maintain several chromosomes throughout a number of division cycles. Hence, the time for recombination would be extended after the fusion. The two parental genomes could potentially serve as the basis for gene expression. These developments would considerably enhance the variety of chemicals used in the molecules that are created by modified cells. The development of novel chemicals, particularly in the realm of antibiotic research, is made possible by these new L-forms [10]–[13].

CONCLUSION

All photosynthetic organisms, including cyanobacteria, have the ability to make secondary metabolites using carbon dioxide and sunlight energy, which is in line with the contemporary idea of sustainable development. Nowadays, cyanobacteria are routinely used for the commercial manufacture of value-added substances, such as particular metabolites and synthetic biochemicals, using metabolic engineering-based techniques. Jeong et AL review,.'s however, discusses a substantial synthetic and systems biology strategy for cyanobacterial advanced metabolic engineering. This requires knowledge of heterologous expression for the generation of cyanobacterial secondary metabolites as well as an overview of the known biosynthetic clusters coding for important substances. As an example, the review emphasises how advances in next-generation sequencing technologies, along with the gathering of omics data such as transcriptome, translatome, proteome, metabolome, or interactome, enrich global databases, creating enormous opportunities to more effectively

manipulate and regulate cyanobacteria productions. The existing limits of cyanobacterial engineering may be easily overcome with the creation of an in silico model at the genome size.

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CHAPTER 24

BIOLUMINESCENCE: A NATURAL DEFENSE MECHANISM IN MARINE ORGANISMS

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ABSTRACT:

The terms "bioluminescence" and "lumen," which both mean light in Greek, are combined to form the phrase. A live creature producing and emitting light as a result of a chemical process in which chemical energy is transformed into light energy is known as bioluminescence. It is a sort of chemiluminescence where light, rather than heat, is released as a consequence of a chemical process catalysed by the enzyme luciferase. The majority of luminescent bacteria occur in saltwater, with the other species living on land or in freshwater. They are the most common kind of light-emitting organism. The majority of luminous bacterial species are found in nature in symbiosis with host organisms, even though most are capable of thriving in freedom. In symbiosis, the host uses the adopted light to communicate, attract prey, and dissimulate itself from predators while the bacteria are fed with conveniently accessible food sources for development. There are, however, certain kinds of luminous bacteria that must coexist with their hosts and need special nutrients that the host can only provide.

KEYWORDS:

Bioluminescence, Bacterial Growth,Natural Defense Mechanism, Microbiology.

INTRODUCTION

According to estimates, 90% of deep-sea marine life emits bioluminescence in some way. Bioluminescent creatures are those that exhibit the bioluminescence process.While these mandatory symbionts have been found, they cannot be cultivated in the lab for further research since they cannot be separated from their host [1]–[3].Most luminous bacteria fall into one of three main genera: Photobacterium, Vibrio, or Photorhabdus. The Photobacterium and Vibrio genera are mostly used to classify organisms that occur in the marine environment, whereas the Photorhabdus genus is used to classify species that dwell on land. The Vibrio species occur both as free-living forms and as symbionts in the sea, in contrast to the Photobacterium species, which are often light organ symbionts of marine creatures.

Many luminous bacteria are parasitic, infecting terrestrial insects like caterpillars (Photorhabdus luminescens) and marine crustaceans (Photobacterium and Vibrio families), respectively. Nematodes serve as the bacteria's intermediary host. The gut and skin surface of practically all marine creatures often have free-living, luminescent bacteria that are disseminated in the saltwater and function as general parasites [4]–[6].The extra-cellular chitinase secreted on the cell wall of all luminous bacteria aids in the breakdown of the ingested chitin for luminous bacteria found in the digestive systems of marine animals.All luminous bacteria are rod-shaped, gram-negative microorganisms with flagella that facilitate motion. Although each species of luminous bacteria differs in a number of properties,

including the precise growing conditions and the reaction kinetics of the luciferase involved in light generation, they all share this trait. Moreover, luminescent bacteria are facultative anaerobes that may continue to thrive even when there is a shortage of molecular oxygen. Although though diverse kinds of luminescent bacteria exhibit physiological variability, all luminescent microorganisms use relatively comparable biochemical machinery to create light. Its light-producing molecular machinery's initiation and energy production are closely controlled by a single signalling channel. The key biochemical elements of bacterial bioluminescence and the orderly molecular interactions/reactions throughout the reaction route are described in the text and in more detail in the following Pictures. The following sections will quickly go through the signalling pathways that allow cellular factors and intercellular communication to precisely regulate the induction and repression of the lighting function in luminescent bacteria. Figure 1 recent developments in bioluminescence technology.

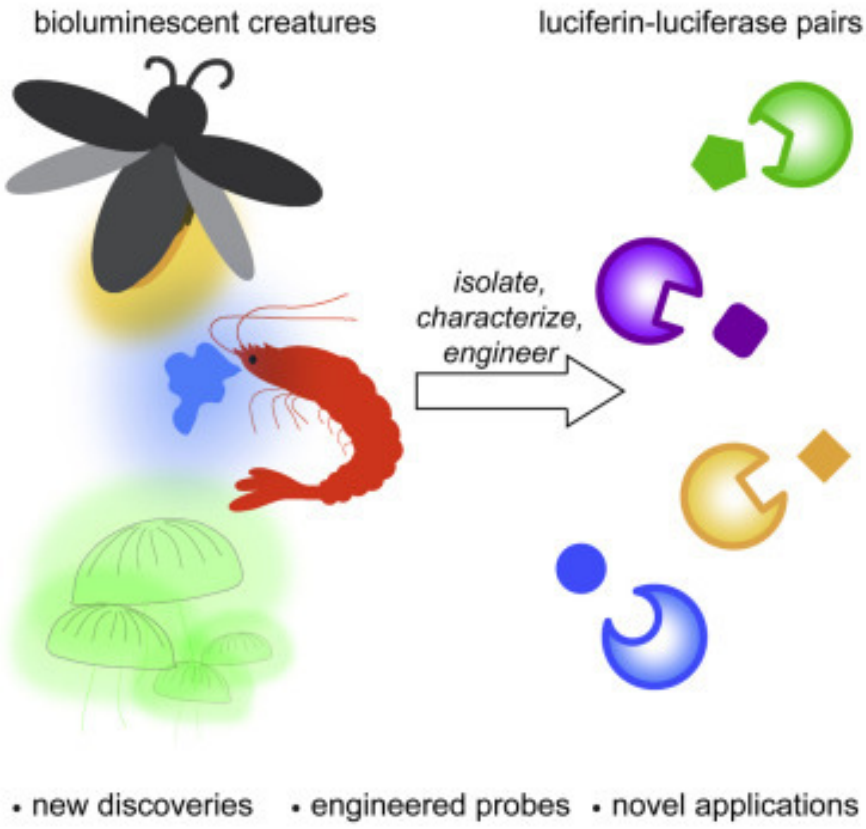

Figure 1: Recent Developments in Bioluminescence Technology [ScienceDirect].

Bacterial luciferase and the Light Color: Biochemistry of the Bacterial Bioluminescence Reaction

At the core of bacterial bioluminescence is the enzyme known as bacterial luciferase, which catalyses light emission. Yet, in addition to bacterial luciferase, the catalytic machinery of luminous bacteria also consists of enzymes that provide and replenish the substrates for bacterial luciferase.The lux genes are the Genetic sequences that encode the proteins in the luminous system. Bacterial luciferase is a heterodimer made up of two distinct polypeptides, alpha and beta. Its beta subunit is thought to serve as a supporting scaffold that aids in the subunit's conformational shift during catalysis.Reduced flavin mononucleotide, molecular oxygen, and long chain fatty aldehyde are the substrates of bacterial luciferase. The extra

energy that results from the simultaneous reduction of molecular oxygen and the oxidation of FMNH2 and aldehyde is released as blue/green light emission. Tetradecanal is thought to be the natural aldehyde utilised in vivo by the luminous bacteria, and luciferases operate effectively with long chain aldehydes of eight carbons or longer.As the excited electron on the flavin chromophore returns to the ground state, the distinctive hue reveals the energy level of the photon that was created. Experts in the area have found that distinct luciferase emission colours were produced by flavin analogues with substituted atoms in the chromophore moiety.

A interesting phenomena known as bioluminescence is when living things produce light as a result of metabolic processes. Marine species frequently exhibit bioluminescence, which is essential to their existence. The bioluminescence that some marine species produce as a natural defence mechanism is examined in this study paper. The publication summarises recent research on the bioluminescence of marine creatures, including its diversity, evolution, and uses. The study emphasises how vital bioluminescence is to marine species' existence, particularly how it helps them find food, reproduce, and build defences. The article also covers the various defence mechanisms used by marine species, such as rapid blasts of light to frighten away predators. The results of this study indicate that bioluminescence is a vital survival mechanism for marine creatures, and that knowing how it works will enable us to create new approaches to environmental monitoring and bioremediation.

DISCUSSION

The colour emission spectrum of bacterial bioluminescence has also been shown to be distorted by point mutations at the flavin chromophore binding site, demonstrating that the distinctive emission colour depends not only on the chromophore that emits the photon but also on the electronic structure of the chromophore-binding microenvironment in luciferase. In addition to luciferase, certain luminous bacteria also have fluorescent proteins that they use to alter the colour of their emission and set them apart from other varieties. Blue-colored photons are produced when the blue fluorescent proteins of Photobacterium phosphoreum and Photobacterium leiognathi interact with their respective luciferases [7]–[9].

Similar to this, the V. fischeri strain Y-1 emits yellow light when the yellow fluorescence protein is present. The kinetics of the lucifrease-catalyzed process are also changed by the presence of fluorescent proteins in bacteria. The capacity of fluorescent protein to interact with the high-energy luciferase reaction intermediate, leading to varied colours of light emission, is supported by the structural/molecular foundation for the shift/change in emission colours. Yet, it is evident that the oxidation of FMNH2, aldehyde, and bacterial luciferase is what provides the energy input. Bacterial luciferase requires constant substrate supply in order to emit light for extended periods of time. The constant light emission in luminescent bacteria must therefore be maintained by several different enzymes continuously producing the substrates for the bioluminescence reaction because the time frame and the amount of product generation in enzymatic reactions are constrained by the availability of the substrates. The lux operon codes for the enzymes that replenish the aldehyde substrate, including the multienzyme complex fatty acid reductase, whose lux genes are located right next to the luxA and luxB genes of luciferase.

Fatty Acid Reductase: Fatty Aldehyde Production and Recycling in Luminous Bacteria

Although a structural model of the fatty acid reductase at atomic resolution is not yet available to visualise how fatty acids are exchanged among subunits of this multienzyme complex and modified towards the fatty aldehyde in three-dimensional space, fatty acid reductase has been divided and isolated into individual subunits, allowing the function of

each part of the protein complex to be determined. A big protein with a molecular mass of around 0 kilodaltons is fatty acid reductase. These big polypeptides make up this complex. While this group of lux genes' assigned letters are C through E, aldehyde production does not develop in the same manner. The transferase, which is encoded by LuxD, transfers the acyl moiety of fatty acyl-ACP, acyl-CoA, and other activated acyl donors produced from the universal fatty acid biosynthesis route to the hydroxyl group of a serine on the transferase. The ester is then hydrolyzed to become a fatty acid. Tetradecanal, the aldehyde employed in the bioluminescent process in vivo, has a high selectivity for acyl derivatives with chains longer than fourteen carbons.

The synthetase then receives the fatty acid that the transferase created and activates the carboxylate function with ATP to create a mixed anhydride of acyl-AMP. After reacting with the reactive thiol of cysteine in the synthetase, the acyl group on the anhydride intermediate, acyl-AMP, forms a covalent bond with the synthetase and releases AMP.The fatty acyl group switches the covalent bonding partner from synthetase to reductase by a reciprocal trans-esterification process between a thiol group on the synthetase and a thiol group on the reductase. The reductase then releases fatty aldehyde by breaking the thiol-ester bond by reduction with NADPH hydride, returning to its free enzyme state. The fatty acid reductase subunits are arranged radially, with a core of four reductase subunits that form a weak combination with each synthetase subunit. The transferases branch off the synthetase at the fatty acid reductase complex's outermost perimeter and rapidly dissociate.

The benefit of such complex formation is that reaction intermediates are efficiently channelled from one subunit to the next without being exposed to the hydrophobic hydrocarbons of the reaction intermediates in the aqueous cytosolic environment of the luminescent bacteria. The material is processed from the aqueous periphery, where the acyl group is received, towards the centre of the complex, where the reductase reduces the fatty acyl group to aldehyde. In addition, the arrangement of transferase, synthetase, and reductase in a radial fashion channels the reaction material through different microenvironments that stabilise the acyl intermediate at various stages of reaction. The ultimate product is tetradecanal because all of the proteins in the fatty acid reductase complex have a strong affinity for tetradecanoyl groups.

FMNH2 - FMN Reductase refuelling

Electron carriers like flavin mononucleotide are found in both prokaryotic and eukaryotic cells' electron transport chains. Via radical production, FMN may give and receive one electron, or it can give and receive two electrons through hydride transfer.Both riboflavin and FMN are common nutrients required for the survival of both eukaryotic and prokaryotic cells, and FMN is generated from riboflavin.The simple insertion of the luxCDABE genes, which encode the bacterial luciferase and the fatty acid reductase complex, into the cell is all that is needed to transform a nonluminous bacterium like Escherichia coli into a light-emitter, proving that FMNH2 is easily available from the electron transport chain in all bacteria. These enzymes are often present in bacteria because riboflavin and FMN production are necessary for bacterial development, even though the biosynthesis of riboflavin and FMN in luminous bacteria is carried out in numerous phases by enzymes that are not represented by the lux gene system. A FMN/NADH oxidoreductase in luminous bacteria catalyses the reduction of FMN to FMNH2 by adding the hydride from NADH to the process.

Recent kinetic studies have been conducted by experts in the area to try to understand how FMNH2 is transferred from the FMN reductase to the bacterial luciferase. Based on the strong binding affinities of FMN reductase towards both FMN and FMNH2, and the tightly

anchored FMN binding geometries in the crystal structure of FMN reductase, investigators have speculated that the transfer of FMNH2 from FMN reductase to bacterial luciferase occurs by transient formation of a luciferase-FMN reductase complex in luminous bacteria, rather than through free diffusion of FMNH2 dispatched into the cytosol. As a consequence, FMN would be recycled and reduced to FMNH2 extremely effectively, ready for utilisation by bacterial luciferase.

Molecular oxygen is the common oxidant in bioluminescence

Molecular oxygen, which comes from the extracellular environment, is a crucial part of the biochemistry of bacterial bioluminescence. Light-emitting bacteria need the presence of molecular oxygen to produce light. The energy expended on the reduction of molecular oxygen to a peroxy reaction intermediate, and finally to water, in the bacterial luciferase-catalyzed reaction, acts as a catalyst for the release of the potential energy from the oxidation of both FMNH2 and fatty aldehyde in the form of photon emission.

The activities involved in the reduction of the molecular oxygen function as an energy sink, draining the reducing power of the substrates. All other biological luminescence systems, excluding bacterial bioluminescence, use molecular oxygen as the necessary oxidant in their luminescence biochemistry. This causes the production of unstable, high-energy intermediates, which release the excited chromophore's potential energy as light. In this context, it may be said that molecular oxygen acts as a key to unlock the energy locked up in FMNH2 and fatty aldehyde for bacterial bioluminescence.

Bacterial bioluminescence is used as a biosensor and a gene expression reporter

By transferring the luxCDABE genes, which turn nonluminescent bacteria into light emitters, it is possible to effectively develop additional bacteria with the ability to catalyse the emission of light and the production of the aldehyde substrate via fatty acid reductase.Researchers now have a simple alternative to assess and identify the development and living circumstances of bacteria thanks to the adaption of light as an accessory characteristic of traditionally nonluminescent bacteria. One of the many techniques used to find dangerous bacteria in sources of human food is the use of bacterial bioluminescence. One may quickly ascertain if a food supply is contaminated with bacteria by cultivating a food sample in the presence of a recombinant bacteriophage bearing the luxCDABE insert.

Quorum Sensing Controls LuxCDABE Expression in Luminous Bacteria

Strict regulatory measures are in place to limit the workload of the lighting-emitting equipment in luminous bacteria. Not only is a high level of luxCDABE gene expression necessary to create bacterial luciferase and fatty acid reductase, but also the synthesis of bacterial luciferase's substrates is necessary to sustain the light emission over extended periods of time. As a result, the habitat and development of luminous bacteria have a significant impact on their ability to emit light. Due to the lack of substrate for the bacterial luciferase reaction and the dormancy of the expression of the luxCDABE genes, luminous bacteria grown in liquid medium at low cell density emit only a little amount of light in the laboratory environment. The quick accumulation of synthesis substrates and enzymes from the activation of the expression of the luxCDABE genes causes the intensity of the light emission to climb substantially from the middle to the end of the exponential growth phase [10].

CONCLUSION

A method similar to an autocatalytic chemical reaction, in which the reaction's products likewise catalyse the reaction from which they were formed, results in the production of more products and, thus, more catalysts, is how the expression of the luxCDABE genes increases. Moreover, the luxCDABE genes' ability to produce light has been used as a reporter of gene expression for research into the regulatory factors that influence RNA polymerase's efficacy in initiation and transcription at various promoters. The structural lux genes may act as a biosensor whose expression will track the presence of hazardous waste in the environment when the luxCDABE genes are controlled by an environmentally regulated promoter. Genetically edited bacteria containing the lux luminous system have been used in the pharmaceutical industry to test the effectiveness of medicines in treating bacterial infections in mammals, with possible human models including mice, pigs, and monkeys. As the bacteria are killed by the antibiotics in this screening procedure, the intensity of luminescence in the infected organs and tissues will decrease; therefore, bacterial bioluminescence serves as an indicator of bacterial growth, allowing the correct antibiotic dosages to be determined and an effective course of treatment to be established.

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CHAPTER 25

CHALLENGES IN MICROBIAL PHYSIOLOGY AND METABOLISM APPLICATIONS

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ABSTRACT:

In order to improve the environmental sustainability of the transportation and chemical manufacturing industries, metabolic engineering may play a critical role. Engineered microbes are already being employed in industrial-scale operations thanks to the field's research and development. Nevertheless, obtaining the titres, yields, and productivities necessary for commercial viability is sometimes difficult. Organisms have developed a wide range of metabolisms and physiologies in an effort to adapt to and flourish in various environments. Its metabolic abundance provides a treasure trove of biochemical processes that researchers may take use of to create a variety of goods with economic value1. In order to create self-replicating biocatalysts, metabolic engineers often transfer genes that encode metabolic enzymes from biosynthetic pathways of interest into genetically tractable microbial hosts. These living cell factories have the ability to manufacture a variety of goods using affordable and renewable resources, which is a significant benefit. These goods include biofuels, common chemicals, biomaterials, and high-value speciality chemicals.

KEYWORDS:

Metabolism Applications, Bacterial Growth,Microorganisms, Microbiology, Microbial Physiology.

INTRODUCTION

The physiological characteristics of the host organism, which may either impose restrictions on designed biosynthetic pathways or, conversely, enhance their performance, are often responsible for determining the efficiency of microbial chemical synthesis. In this Article, we cover many features of microbial physiology that often pose challenges for metabolic engineering and provide workarounds. We also discuss numerous cases in which physiological characteristics of hosts, whether natural or designed, have been used to support engineered metabolic pathways for chemical synthesis [1]–[3].Because engineered microorganisms transform renewable biological resources, waste streams, and even CO2 into the energy, chemicals, materials, food, and medicines that power the global economy, metabolic engineering will play a crucial role in our transition from a petroleum-based economy to a new biobased economy. In order to tackle climate change and environmental deterioration, such a bioeconomy has the potential to reduce greenhouse gas emissions and facilitate the development of sustainable industrial techniques.

A crucial initial step is demonstrating the functional expression of biosynthetic pathways in microbial hosts, although this is often insufficient to produce strains suitable for commercial production. Generally, in order to boost metabolic flow towards the desired product and reduce byproducts, it is also essential to rewire the native metabolism by up-regulating,

down-regulating, modifying, or deleting endogenous genes. For the generation of resilient and extraordinarily prolific strains, it is also essential to integrate the designed metabolism with natural host physiology. Overexpressed biosynthetic pathways may affect the microbial host in a good or negative manner. The native physiology, on the other hand, may bring challenges or advantages to the targeted biosynthetic route. To achieve the robustness, productivity, and ultimately overall performance needed of high-producing strains, it is crucial to understand and manipulate the relationships between metabolism and host physiology.In this Article, we discuss the key elements of cellular metabolism, cell development, and subcellular architecture that may affect designed metabolic pathways. We provide examples of the significance of several physiological characteristics in the design of microbial cell factories and discuss how these characteristics might either create problems or possibilities for the creation of production strains [4]–[6].

Tolerance And Toxicity in Cells

Chemical synthesis may be hampered by a toxic buildup of chemicals in the medium or in the cell, which can reduce cell viability and growth. Nevertheless, the ways in which feedstocks, substrates, products, intermediates, or byproducts cause cellular toxicity as well as the ways in which microbial hosts develop a tolerance to these substances and how toxicity impacts production varied greatly. Chemical sensitivity and tolerance are complicated physiological qualities involving several genes that interact dynamically in response to environmental changes, making it difficult to fully understand these processes. Damage to biological molecules, physical alterations and damage to cell membranes, production of stress responses, changes in metabolic activity, and increased metabolic load are some of the toxicity processes with the greatest understanding. Systems biology, adaptive laboratory evolution, and other non-directed methods like genome shuffling and random mutagenesis have all been used in attempts to clarify these pathways.

Genetic interactions involved in the processes of toxicity and microbial resistance to severe circumstances may be revealed through systems biology investigations. It is possible to gain insights into the cellular damage caused by chemical or physical stresses and the adaptive mechanisms that microorganisms have evolved to respond to them by integrating global quantitative information at the genomic, transcriptomic, proteomic, or metabolomic level. This can lead to possible strategies to engineer organisms with increased tolerance to toxic conditions, products, feedstocks, or metabolites.The mechanism of toxicity of isopentenyl pyrophosphate, an isoprenoid precursor that decreases cell growth and productivity when accumulated in engineered strains, was recently studied in Escherichia coli, and the results are well demonstrated. Increased levels of IPP reduce food absorption, lower ATP pools, and perturb nucleotide metabolism, which implies a stop in overall metabolism, according to metabolomic and transcriptome data. These findings concur with the accumulated levels of the nucleotide analogue AppppI, which has been associated with cell death9. In a more recent genomic and transcriptome investigation on yeast tolerance to branched-chain alcohols, it was shown that the cell interprets these substances as signals of nitrogen starvation, which inhibits growth. Deletion of the nitrogen catabolite repression regulator gene GLN3 may reverse this adaptive response, promoting cell proliferation and raising isobutanol synthesis. In general, rational engineering attempts to promote cell tolerance may be guided by the underlying knowledge of the processes of toxicity and cell response acquired from systems-level investigations.

Although non-rational techniques like ALE, random mutagenesis8, and genome shuffling may more quickly produce desired phenotypes, systematic, omics-based approaches can provide molecular insights that might assist design tolerance. These techniques include

subjecting organisms to circumstances that are inhibitory but not deadly in order to enhance tolerance, then choosing mutants that are enriched in the population. Once the population contains mutants with the necessary tolerance, selective pressure is progressively enhanced. The genetic foundation for the improved traits may still be at least partly understood by sequencing the chosen mutants and augmenting the data using bioinformatics and multi-omics techniques, even if these approaches do not directly unveil mechanisms of toxicity or tolerance.

For instance, sequencing yeast cells from ALE experiments with higher tolerance to 3 hydroxypropionic acid showed that this trait was caused by a mutation in the bifunctional dehydrogenase gene SFA1. Transcriptomic analysis revealed that the acquired tolerance is also influenced by the differential expression of genes related to oxidative stress and redox balance, with Sfa1p eliminating hydroxypropionic acid through a glutathione-dependent mechanism. These unfocused methods have been used in several research to enhance and better understand cells' tolerance to a variety of difficult circumstances, including as high temperatures, low pH, and hazardous compounds such organic acids and alcohols1. No matter how strain tolerance is raised, this phenotype does not always translate into higher chemical output. A yeast strain with higher fatty acid tolerance was recently shown to improve medium-chain fatty acid synthesis by up to 1.7 times. In contrast, boosting an E. coli strain's tolerance to free fatty acids did not improve its productivity, pointing to the existence of additional regulatory mechanisms. A more notable example was the rise in production in yeast strains with the cytosolic but not the mitochondrial isobutanol pathway when GLN3 was deleted to promote isobutanol tolerance. While the reason is unclear, it probably relies on the mechanism by which tolerance is developed, how this mechanism interacts with the biosynthetic route, and how much of a bottleneck product toxicity is at a particular titre [7]–[9].

DISCUSSION

Utilizing unusual organisms naturally adapted to survive or even thrive under harsh conditions is an appealing alternative to conventional organisms for the development of new industrial processes, even though increasing the tolerance of established hosts preserves all the benefits they have to offer. Established hosts are typically less able to withstand and maintain high productivity in inhospitable conditions that are frequently found in industrial bioreactors than microorganisms with physiological adaptations that allow them to grow under conditions like high temperatures, extreme pH, high salinity, and toxic chemicals-. This has the potential to increase efficiencies and reduce costs. Also, these organisms naturally generate a wide variety of interesting enzymes and products, which has spurred research to better understand their physiologies and create genetic engineering tools for them. Halophiles, thermophiles, acidophiles, and alkaliphiles are some of the extremophiles that have been studied the most potential biotechnological uses.

High salinity settings are ideal for halophiles, which allows for fermentations using seawater rather than freshwater. Due to its high salinity, the use of saltwater facilitates the preservation of sterile conditions in addition to boosting the sustainability of bioprocesses. In order to boost the production and scalability of these biodegradable polymers, attempts have been made to genetically modify halophilic bacteria, such as Halomonas bluephagenesis, to accumulate polyhydroxyalkanoates. Additional halophiles have been developed to produce medicines, enzymes, and biofuels, these early instances show how modified halophilic microbes might make it possible for seawater fermentations to produce useful products [10], [11].

Thermophiles, or organisms that have evolved to live in hot environments, are also of interest to biotechnological research. Fermentations carried out at higher temperatures may aid in the extraction of volatile compounds, save cooling costs, and improve sterility. In addition, lignocellulosic biomass is spontaneously degraded and consumed by numerous thermophiles, a process that is similarly favoured at higher temperatures. These characteristics make thermophiles suitable for consolidated bioprocessing, which involves simultaneous fermentation, biomass saccharification, and biomass hydro- lases generation to boost efficiencies and lower costs. The synthesis of biofuels, such as butanol and ethanol, has been proven utilising consolidated bioprocessing, for instance, employing the thermophiles Clostridium thermo-cellum, Thermoanaerobacterium saccharolyticum, and Caldicellulosiruptor bescii-.

It has also been shown that organisms modified to endure severe pH conditions are acceptable for industrial uses. There are several acidophilic bacteria, some of which are also able to survive in hot environments.They are ideal for use in lignocellulosic biorefineries, as well as in the food and textile sectors, due to these characteristics. Some acidophiles, such Acidithiobacillus thiooxidans, have the ability to oxidise and solubilize metal sulphide minerals, making them perfect for biomining. For instance, bioleaching and biooxidation are sustainable methods that may be used to extract metals from ores that include sulphides. Moreover, natural sources of enzymes utilised in the production of detergents, antibiotics, medicines, and food are thought to include severe pH alkaliphiles. As recently shown by a Bacillus marmarensis strain modified to manufacture ethanol from wastewater polluted with alkaline algae, these organisms have also been used to make bioplastics and biofuels. Hence, these extremophiles may facilitate bioprocesses that take use of varied water sources, extreme pH levels, or the distinctive enzymes these organisms create.

Extremophiles are still not widely used in bioprocesses due to our incomplete knowledge of their physiologies and the lack of tools for their genetic modification,Industrial microbiology has developed because to tamed microbes' innate capacity to consume simple carbohydrates and starch. Concerns concerning sustainability and rivalry with food production have been raised, nevertheless, as a result of this industry's potential development. A more sustainable option is lignocellulosic biomass, which is made from the inedible sections of crops used for food or as energy sources. Nevertheless, lignocellulose has evolved to be structurally strong and protect plants, making it more resistant to microbial deterioration and, thus, more bioprocess assimilable. Moreover, lignocellulose has a far more varied composition than starchy and sugar crops made of glucose and fructose, including differing levels of acetyl groups as well as various hexoses, pentoses, and phenolic chemicals.

These varying lignocellulose compositions pose particular difficulties. For example, not all microbes can assimilate xylose; those that can still need engineering to allow simultaneous co-utilization of these sugars for increased process efficiency, Adaptive carbon catabolite repression mechanisms or competition amongst sugar substrates for cellular importers are examples of physiological characteristics that normally prevent co-utilization. Genetic engineering has been used to alleviate the inhibition of xylose carbon catabolism, for example by altering a hexose transporter to eliminate glucose inhibition or by diverting xylose absorption via the tricarboxylic acid cycle, which is distinct from glycolysis. ALE has also shown to be a successful tactic, as demonstrated by the forced co-consumption of glucose and xylose. As an alternative, coculture systems, in which several strains are designed to preferentially utilise one substrate, may be used to bypass carbon catabolite suppression altogether.

Since lignin's phenolic compounds hinder cell development and are tough to digest, lignin valorization is considerably more difficult. Discovering bacteria that can thrive in lignin-derived chemicals and transform them into useful products is of increasing interest. Recent research has focused on Pseudomonas putida KT, Sphingobium sp. SYK-6, and Rhodococcus opacus PD0. Established microbial hosts may also acquire the capacity to transform lignin derivatives into useful chemicals [12], [13].

It is usually possible to show that biosynthetic pathways are functioning in microbial hosts just by expressing active enzymes and sometimes by deleting certain endogenous genes. To go from proof-of-concept strains to production strains with commercial significance, strains must be improved. To do this, the interactions between altered metabolic pathways and host physiology must be given more thought. Three different levels may be identified at which host metabolism and physiology may be synchronised to improve yields, titres, and productivities as a result of the physiological possibilities and difficulties highlighted in this review. The host physiology may, at the very least, be chosen or developed to withstand the toxicity of the substrates, products, intermediate metabolites, or fermentation conditions. Intermediate stages of coordination include modifying the host physiology to make up for metabolic, energy, or redox imbalances brought on by engineering the metabolism with the goal of restoring cellular homeostasis, preserving healthy cell development, and enabling high product yields. Eventually, when the host's metabolism and physiology work together to produce cells that are resilient and productive, the highest degree of coordination is reached. High yields exist at this level due to the host's expression of well-balanced metabolic enzymes as well as the fact that the designed route has evolved into a selective one; the cell would suffer if production were to stop. Hence, increasing our comprehension of microbial physiology and our capacity to design it will enable us to fully use the potential inherent in microbial hosts and circumvent the major challenges limiting their output.

CONCLUSION

The potential of microbial fermentations has been increased thanks to metabolic engineering, and it will continue to play a crucial part in our pursuit of renewable energy, environmentally friendly manufacturing, and the realisation of a future bioeconomy. By logically integrating enzymes from various animals to develop de novo metabolic pathways2, metabolic engineering has recently shown promise beyond the synthesis of chemicals naturally generated in life to attain the synthesis of synthetic compounds. In the pharmaceutical business, where new therapies are continually sought after, or in the creation of chemicals and biomaterials with unique features, this insight has the potential to unleash new, high-value goods. In this sense, the design, integration, and optimization of these pathways in the host of choice will be significantly aided by mining for new enzymes capable of executing complex chemistries in conjunction with the use of innovative methods for computational retrobiosynthesis.

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