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ADVANCED TOOLS & TECHNIQUES IN MICROBIOLOGY

Edited By
Dr. S. P. Dwivedi

Advanced Tools & Techniques in Microbiology

COMPILED AND EDITED BY

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Advanced Tools & Techniques in Microbiology

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Preface

Welcome to the open access publication of our book Advanced Tools & Techniques in Microbiology. The goal of this book is to provide students with more affordable, high-quality learning resources that nevertheless uphold the strictest academic standards. You may share, remix, and build upon the material of this book under the terms of the Creative Commons Attribution 4.0 International (CC BY) licence, as long as you attribute Ennoble Books and Publications and the authors of the original work. You are free to utilise the complete book or just the parts that are most pertinent to the requirements of your course because our books are openly licenced. By giving your students specific chapters and elements of your syllabus in the order you see fit, you can remix the content. Even better, you may include a direct link in your syllabus to each portion of your book's online version.

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About Advanced Tools & Techniques in Microbiology

The scope and sequencing requirements for a Microbiology course for non-majors are covered in this book. The fundamental ideas of microbiology are presented in the book, with an emphasis on how they apply to allied health professions. The didactic aspects of microbiology keep the career-application focus and scientific rigour inherent in the subject matter while making the topic engaging and approachable for students. This book's scope and order were prepared and reviewed with help from a variety of academic educators. It is created to satisfy the requirements of the majority of microbiological courses for allied health students and non-majors. We have also taken into account the needs of universities that provide microbiology to a mixed audience of science majors and non-majors by commonly combining themes like environmental and applied microbiology and the history of science that might not have clear clinical relevance.

The content of this textbook has been organised with these goals in mind, progressing logically from basic to more sophisticated concepts. The introductory chapters give a general overview of the field, with individual chapters focused on microscopy, cellular biology, and every type of microorganism. Students then investigate the fundamentals of microbial biochemistry, metabolism, and genetics, subjects that serve as a foundation for comprehending the many strategies for regulating and thwarting microbial growth. Focusing on how interactions between microorganisms and the human immune system affect human health and disease, the introduction chapter is followed by a chapter on microbial pathogenicity. The text's final several chapters offer an overview of medical microbiology and

list the traits of microbial illnesses in alphabetical order by body system. The Table of Contents has been carefully crafted to meet the needs of our readers, but some teachers may want to present the material in a different sequence.

Microbiology has the advantage that educators can modify it to fit the teaching style that works best in their classroom. To research and comprehend the metabolic processes, genetics, functions, and interactions of bacteria with other creatures, a number of microbiological techniques and procedures have been specially developed over the years. These microscopic creatures are mostly studied using methods for cultivating, identifying, isolating, staining, and engineering. Additionally, they have uses in molecular biology, genetics, plant physiology, and other branches of biological research. Additionally, while some of them improve our health, others can lead to fatal diseases. Others are employed in the production of foods and beverages, and we must study these species in order to comprehend everything. Given the wide range of applications for microbiology and its fundamental methods, this book also provides an overview of the fundamental methods employed in microbiological laboratories.

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January 2023

CHAPTER 1

Microscopy Techniques

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Microbiology is the study of microbes, that are tiny unicellular or cell-cluster organisms. This contains both eukaryotes (fungi as well as protists) and prokaryotic cells. Viruses are also examined, despite the fact that they are not properly classified as living beings. Microbiology, in a nutshell, is the study of life and creatures that are too tiny to be seen by the naked eye. Immunology, or the study of the immune system, is often included with microbiology. Immune systems interact with harmful bacteria in general; the two areas often overlap, that's why many universities offer a combined degree such as "Microbiology and Immunology."

Microbiology is a vast field that encompasses mycology, virology, parasitology, bacteriology, and other disciplines. A microbiologist is a microbiology expert. Microbiology is extensively investigated, and the science is always progressing. Researchers have probably only investigated roughly 1% of all microbial species on the planet. Despite the fact that bacteria were first discovered over three hundred years ago, the study of microbiology is still in its infancy in comparison to older scientific disciplines such as biology and botany.

Microbiology (from Greek μῑκρος, mīkros, "small"; βίος, bios, "life"; and -λογία, -logia) is the study of microorganisms, which are unicellular or cell-cluster microscopic organisms. This includes eukaryotes such as fungi and protists, and prokaryotes. Viruses, though not strictly classed as living organisms, are also studied. In short; microbiology refers to the study of life and organisms that are too small to be seen with the naked eye. Microbiology typically includes the study of the immune system, or Immunology. Generally, immune systems interact with pathogenic microbes; these two disciplines often intersect which is why many colleges offer a paired degree such as "Microbiology and Immunology". A wide phrase, "microbiology" encompasses virology, mycology, parasitology, bacteriology, and other subfields.

A microbiologist is an expert in the field. The study of microbiology is actively pursued, and it is a growing area. Of all the microbial species on Earth, we have probably only researched roughly one percent of them. Despite the fact that microorganisms were first directly seen more than 300 years ago, the study of microbes is still relatively young compared to other biological fields like zoology and botany. History Early Before microbes were actually discovered in the 17th century, their presence was theorised for many centuries. Susruta, an ancient Indian physician, blamed germs for a number of ailments about 600 BCE and described in his book Sushruta Samhita that they may spread by touch, water, or the air. Marcus Terentius Varro, a Roman scholar, developed theories about microorganisms in his book On Agriculture, which includes a warning against establishing a settlement close to a swamp because there are breeds of small creatures that cannot

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be seen with the naked eye that float in the air and enter the body through the mouth and nose and cause serious illnesses. According to this paragraph, the ancients were aware that illnesses may be transmitted by as-yet-undiscovered species. Ab Al ibn Sn (Avicenna) said in The Canon of Medicine (1020) that body discharge is first tainted by vile alien worldly bodies before being sick. He also made conjectures on the contagiousness of infectious illnesses like TB and advocated the usage of quarantines to stop the spread of infectious diseases. Ibn Khatima proposed the theory that infectious illnesses are brought on by "minute bodies" that enter the human body and create sickness when the Black Death bubonic plague struck al-Andalus in the 14th century. Girolamo Fracastoro suggested in 1546 that infectious agents that might spread via direct or indirect touch or even without contact across large distances were to blame for epidemic disorders. These early assertions on the presence of microbes were all hypothetical in nature and unsupported by any evidence or scientific research. Before the 17th century, microorganisms were neither confirmed and seen nor precisely and accurately characterised. The reason for this was that none of these early investigations used the microscope, which is essential for microbiology and bacteriology to function as a science.

The majority of microorganisms are unicellular and so tiny that they cannot be seen without artificial amplification. However, there are some multicellular creatures that are minuscule and some unicellular microorganisms that are visible to the human eye. Without a microscope, an item must be roughly 100 micrometres in diameter, however the majority of microorganisms are far smaller than that. Consider the fact that a normal mammal cell is still tiny while being around 10 m wide. In comparison to bacteria, which have cells that are approximately 1 m in size, viruses may be 10 times smaller. The sizes of different things are shown by a bar down the bottom. Unusually little at 1 mm from the egg is located at the far right. A human egg and a pollen particle, both measuring around 0.1 mm, are to the left. A typical plant or animal cell follows, measuring between 10 and 100 m. A red blood cell follows at slightly about 10 m. A mitochondrion and bacterial cell follow at a distance of about 1 m. A smallpox virus follows at around 500 nm. A flu virus follows at a distance of around 100 nm. A polio virus follows at a distance of around 50 nm. Proteins follow, with sizes between 5 and 10 nm. Lipids are the next, with a size range of 2–5 nm. The next molecule is C60, a fullerene molecule that is around 1 nm long. And last, an atom is 0.1 nanometers in size. The use of light microscopes to examine objects bigger than 100 nm (the size of a flu virus). Materials 1.5 nm (bigger than an atom) and smaller may be studied with electron microscopes (the size of many bacteria). The average virus is roughly 100 nm in size, 10 times smaller than the average bacteria (1 m), which is at least 10 times smaller than the average plant or animal cell (10–100 m). For an item to be seen without a microscope, it must be roughly 100 m in size.

In addition to size, microorganisms vary from one another in terms of their structure, environment, metabolism, and a wide range of other traits. There are numerous multicellular creatures that are too tiny to be seen without a microscope, despite the fact that we commonly conceive of microbes as being unicellular. even acellular organisms, such as viruses, exist (not composed of cells). Each of the three kingdoms of life—Archaea, Bacteria, and Eukarya—contains microorganisms. Bacteria and Archaea include only prokaryotes (cells without a nucleus), while the category Eukarya contains only eukaryotes (their cells have a nucleus). The three domains of life do not apply to all microbes, including viruses. We will briefly describe each of the major categories of microorganisms in this section. The many species that make up each category will be covered in more detail in later chapters.

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Prokaryotic Microorganisms

Nearly every ecosystem on earth has bacteria, even within and on top of people. The majority of bacteria are beneficial or harmless, however some are pathogens that may damage both people and other animals. Because DNA does not reside within a proper nucleus, bacteria are considered prokaryotic. Peptidoglycan is found in the cell walls of the majority of bacteria. The overall form of bacteria is often used to characterize them. Spherical (coccus), rod-shaped (bacillus), or curved are typical shapes. Bacillus has a rod form. The shape of Vibrio is a comma. A long, oval-shaped Coccobacillus is present. A stiff spiral is a Spirillum. A flexible spiral is a spirochete. They can develop in a variety of habitats and use various nutrition combinations because they have a broad range of metabolic capacities. Some bacteria utilise light energy from the sun to grow and fix carbon dioxide, including oxygenic cyanobacteria, anoxygenic green sulphur bacteria, and green nonsulfur bacteria. Non-photosynthetic bacteria get their energy from organic or inorganic substances in their surroundings.

Archaea are prokaryotic single-celled creatures. In addition to having distinct evolutionary histories, archaea and bacteria also vary greatly in terms of their genetic make-up, metabolic processes, and the nature of their cell walls and membranes. While the cell walls of most bacteria do not contain peptidoglycan, those of archaea often do, and this material is known as pseudopeptidoglycan. In practically every habitat on Earth, even extreme ones that are very cold, hot, basic, or acidic, archaea may be found, much like bacteria (Figure 1.14). The human body contains some archaea, however none have been identified as human pathogens.

Eukaryotic Microorganisms

All eukaryotes, whether they have one cell or many, including protists, fungi, plants, and mammals, fall within the category Eukarya. Eukaryotes are characterised primarily by the presence of nuclei in their cells.

Protists

Protists are an ad hoc classification of eukaryotes that are not fungi, animals, or other living things. Protists include all protozoa; some algae are protists and some are bacteria. The majority of protists found in algae (plural: algae) are unicellular or multicellular and vary greatly in size, appearance, and environment. Cell walls comprised of cellulose, a form of carbohydrate, enclose algal protists. Algae are photosynthesis-capable creatures that absorb solar energy and emit oxygen and carbohydrates into their surroundings. Cyanobacteria, a class of bacteria, are also referred to as algae, but since they are bacterial prokaryotes, they have a cell wall made of peptidoglycan rather of the cellulose seen in algal protists. Algae play a significant role in many ecosystems because other creatures may generate energy from the waste products produced by all types of algae. Carrageenan and alginic acid, which may be found in certain brands of ice cream, salad dressing, drinks, lipstick, and toothpaste, are among the components present in many consumer goods. The microbiology lab also makes extensive use of an algal derivative. Agar, a gel made from algae, may be used to cultivate microorganisms in a Petri dish by combining it with different nutrients. Algae are also being researched as a potential biofuels source.

Protozoa**,** also known as protozoans, are protists that serve as the foundation of several food webs by supplying essential nutrients to other species. Protozoa come in a wide variety. Some protozoa use flagella, which resembles a whip, or cilia, which resemble hairs to move. Others use their

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cytoplasm and a portion of their cell membrane to move ahead. Pseudopods, sometimes known as "false feet," are these cytoplasmic appendages. Some protozoa feed on organic matter while others are photosynthetic. Some are self-sufficient, while others are parasitic and can only exist by obtaining nutrition from their host. The majority of protozoa are benign, but some of them are diseases that may damage people or animals.

Eukaryotes include fungi (plural: fungus). Although certain multicellular fungi, like mushrooms, resemble plants, they are quite different from them in reality. Since fungi lack the ability to photosynthesize, chitin, as opposed to cellulose, often makes up their cell walls. Yeasts are unicellular fungus, which are covered in the field of microbiology. More than a thousand species have been identified. Yeasts may be found in a wide range of settings, including the human navel and the deep oceans. Some yeasts are useful for making things like bread rise and drinks ferment, but they may also make food go bad. Some even contribute to disorders like oral thrush and vaginal yeast infections. A yeast-like unicellular fungus is called Candida albicans. It is the root cause of oral thrush, a mouth-based yeast infection that typically affects young children, as well as vaginal yeast infections. C. albicans has characteristics with coccus bacteria in terms of shape, although yeast is a much bigger eukaryotic cell (notice the nuclei).

Microbiologists are also interested in moulds, which are multicellular creatures. Long filaments that form apparent colonies make up moulds. Mold may be found in a variety of places, including damp bathroom corners, decaying food, and dirt. Molds are essential to the breakdown of dead plants and animals. Some moulds may induce allergies, while others can develop mycotoxins, which can lead to sickness. Pharmaceuticals like penicillin, one of the most often prescribed antibiotics, and cyclosporine, used to prevent organ rejection after a transplant, have both been produced using moulds.

Helminths

As the majority of helminths are big enough to be seen without a microscope, they are not considered to be microorganisms. However, since the illnesses brought on by helminths include tiny eggs and larvae, these worms belong within the umbrella of microbiology. The guinea worm, or Dracunculus medinensis, is an example of a helminth. When the worm emerges from the skin, it may lead to nausea, vomiting, diarrhoea, and severe sores on the legs and feet. When someone consumes water that has water fleas infected with guinea worm larvae, an infection usually results.

Viruses

Since viruses are acellular microbes, they do not include cells. A virus essentially consists of proteins and genetic material that is inactive outside of a host organism—either DNA or RNA, but never both. However, viruses are able to co-opt the biological processes of the host to reproduce and infect more hosts by integrating themselves into a host cell. The study of all varieties of microorganisms falls under the wide umbrella of microbiology. However, in reality, microbiologists often focus on a single area. For instance, the study of bacteria, fungus, and protozoa is known as mycology; the study of helminths and other parasites is known as parasitology; and the study of viruses is known as virology. Because host-pathogen interactions are essential to our comprehension of the mechanisms behind infectious illness, the study of immunology, or the immune system, is often included into the study of microbiology. Additionally, microbiologists might choose to specialize in a particular branch of microbiology, such as clinical, environmental, applied, or food microbiology.

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The first microbiologist and first to use a microscope to examine microbes was Antonie van Leeuwenhoek. is referred to be the "Father of Microbiology." Although he did not create the microscope, he significantly improved it. Using a single-lens microscope of his own creation, Antonie van Leeuwenhoek made the first observations of modern bacteria and other microbes in 1676. By accomplishing this, Leeuwenhoek established the scientific disciplines of bacteriology and microbiology and achieved one of the most significant discoveries in biology. Ehrenberg coined the term "bacterium" considerably later, in 1828, and it is taken from the Greek v, which means "little stick." Although Van Leeuwenhoek is often credited with being the first microbiologist, Robert Hooke really made the first observation of microbes in 1665 when he saw the fruiting bodies of moulds. Ferdinand Cohn, a botanist who studied photosynthetic bacteria and algae, is usually credited with founding the study of bacteriology (later a subdiscipline of microbiology). He also described various bacteria, including Bacillus and Beggiatoa. A system for the taxonomic categorization of bacteria was initially developed by Cohn.

Cohn's colleagues Louis Pasteur and Robert Koch are often regarded as the pioneers of medical microbiology. The tests planned by Louis Pasteur to refute the then-dominant hypothesis of spontaneous generation cemented the field of microbiology's status as a biological science. Koch is best known for his contributions to the germ theory of disease, which established that particular diseases were caused by specific pathogenic microorganisms. Pasteur also developed methods for food preservation (pasteurization) and vaccines against a number of diseases such as anthrax, fowl cholera, and rabies. He created a set of standards that are today referred to as the Koch's postulates. One of the first researchers to concentrate on bacterial separation in pure culture, Koch described a number of unique species, including Mycobacterium TB, the bacterium that causes tuberculosis. Although Pasteur and Koch are sometimes credited as the founders of microbiology, their research did not adequately portray the entire richness of the microbial world due to their sole emphasis on microbes that directly affected human health. The actual scope of microbiology was not realised until the late 19th century with the work of Martinus Beijerinck and Sergei Winogradsky, the inventors of general microbiology (an earlier term embracing characteristics of microbial physiology, diversity, and ecology). In addition to discovering viruses, Beijerinck also created enrichment culture methods, which together represent two of the field's most significant accomplishments.

While his work on the Tobacco Mosaic Virus helped to establish the fundamentals of virology, it was his invention of enrichment culturing, which made it possible to cultivate a large variety of bacteria with vastly divergent physiologies that had the most direct influence on microbiology. The idea of chemolithotrophy, which Winogradsky initially introduced, helped to highlight the crucial part these three microorganisms play in geochemical processes. He was in charge of isolating and describing nitrifying and nitrogen-fixing microorganisms for the first time. Fields Several subdisciplines within the science of microbiology may typically be classified: Microbial physiology is the study of the biochemical operations of the microbial cell. Includes research on the growth, metabolism, and cell structure of microorganisms. Microbial genetics is the study of the organisation and regulation of genes in bacteria in relation to cellular processes are closely associated with molecular biology

Cellular microbiology is a field that combines cell biology with microbiology Microbiology in medicine is the study of harmful microorganisms and how they affect human sickness. Along with studying disease pathology and immunology, this field also examines microbial pathogenesis and epidemiology. Veterinary microbiology is the study of how bacteria affect animal taxonomy or

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veterinary medicine. Environmental microbiology is the study of how different types of bacteria behave in their natural settings. Includes the study of geomicrobiology, microbial diversity, microbial diversity-mediated nutrient cycling, and bioremediation. Defining important bacterial habitats including the phyllosphere and rhizosphere, soil and groundwater ecosystems, open seas, or harsh conditions (extremophiles).

Evolutionary microbiology is the study of microbial evolution, including research on bacterial taxonomy and systematics. The employment of microorganisms in industrial processes is known as industrial microbiology. Examples include wastewater treatment and industrial fermentation are strongly connected to the biotechnology sector. Brewing is another key use of microbiology in this area. Aero microbiology is the study of microorganisms that are in the air. Food deterioration and disease-causing bacteria are the subject of food microbiology. Producing food with the help of microbes, such as via fermentation. Pharmaceutical microbiology is the study of the microbes that contaminate and ruin pharmaceuticals. Microorganisms that are crucial to agriculture are the subject of agricultural microbiology.

Fishery Microbiology: The study of a huge variety of tiny, unicellular, and mainly unevolved life from microscopic living organisms and how they affect fish and fish culture falls within the purview of the scientific field known as "fisheries microbiology."

Microscopy

The terms "micros," which means "small," and "skopein," which means "to see," are taken from the Greek. Microscopy is the study of using a microscope, which is a tool used to investigate objects that are too tiny to see with the human eye. To put it simply, it is the act of enlarging tiny items so that we may examine them. This fundamental idea has spawned a myriad of ways and tactics for paying attention to little things. The term "microscopy" first appeared around the year 1000 AD when letters were magnified using a glass sphere. While "Iqbal al Haytham" authored a book on "Optics" in 1021 that advanced our understanding of how light functioned, "Hans and Zacharia Janssen" didn't create the first microscope until 1590. The compound light microscope is said to have been created by Galileo in 1609, although Giovanni Faber did not give it that name until 1625.

In 1874, "Ernst Abbe" developed a formula that made it possible to calculate the high resolution of a microscope. Based on a Sziland idea, Ruske and Knoll created the first "Transmission Electron Microscope" in 1931. The nineteenth and early twenty-first century saw advancements in every area of microscopy. The "Nobel Prize" for microscopy has been awarded twice; in 1986, it was split between Ruske and Binig and Rohrer for their work on scanning and tunnelling microscopy and the electron microscope, respectively. The award was granted to Betzig, Hell, and Moerner in 2014 for creating super fluorescence microscopy, which allows for resolution as low as two micrometres.

One of the many varieties of optical microscopy is the compound microscope. This kind of microscope, which is perhaps the most common, consists of a tube with an eyepiece lens on one end and one or more objective lenses of different strengths on the other end that may be changed depending on the item being studied. A platform on which to place the sample, a focusing apparatus, and a source of light shining through the sample from below will also be present. Objects are often only able to be magnified by around 1000 times using optical microscopy. There are many different varieties of optical microscopes, some of which have a single eyepiece or two,

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different light sources, such as light emitting or light reflecting elements, and so on. Additionally, it is doable to have a digital microscope or even computer-displayed camera add-ons. Careful planning is necessary since the conventional method requires shining a source of light onto the sample.

- The sample is lighted from above and the lenses are positioned below when using an inverted microscope. This kind of microscopy is very useful in biological research.
- In stereo microscopy, two identical microscopes are put side by side to provide a single view of the material for each eye. They are used in dissection, the movement of microscopic tools, and the examination of electrical components.
- In polarising microscopy, a polarising filter that allows for the transmission of just one wavelength of light is integrated into the microscopes. Using this specific kind of microscopy, asbestos fibres may be found and examined in crystals.

Metallographic microscopy is used when a light source shines on an opaque substance and is reflected back into the microscopes for study. In forensic and diagnostic microscopy, this method is used. Reflecting microscopes employ concave and convex mirrors to magnify images captured in the infrared, visible, and ultraviolet spectrums. There are other interference techniques that monitor light interference as it passes through a material, therefore this is not an exhaustive list of optical microscopy types. Samples could also undergo further processing, such as the addition of dye or fluorescent compounds, to enhance the image or draw attention to certain components that need more research. The optical microscope, often known as a light microscope, is a kind of microscope that typically makes magnified pictures of tiny objects using visible light and a set of lenses. The first kind of microscope is an optical one, which may have been created in the 17th century in its current compound form. Even though many complicated designs strive to enhance resolution and sample contrast, basic optical microscopes may be rather simple. The item is set up on a stage and may be seen up close using one or both microscope eyepieces. In high-power microscopes, both eyepieces normally display the same picture, but in a stereo microscope, a 3-D illusion is produced using slightly distinct images. There are many different methods to light the sample. Solid objects may be lighted with light coming through the objective lens (bright field) or surrounding it (dark field), while transparent objects can be lit from below. To identify the crystal orientation of metallic objects, utilise polarised light. Phase-contrast imaging may be used to draw attention to minute features with different refractive indices, hence enhancing picture contrast.

Typically, a turret is used to install a variety of objective lenses with various magnifications, enabling them to be rotated into position and offering the option to zoom-in. Due to the limited resolving power of visible light, optical microscopes generally have a maximum magnification power of roughly 1000x. Alternatives to optical microscopy that do not utilise visible light include scanning electron microscopy, transmission electron microscopy, and scanning probe microscopy and as a consequence, may attain significantly higher magnifications even though bigger magnifications are achievable.

Types

Simple microscopes and compound microscopes are the two fundamental kinds of optical microscopes. A straightforward microscope magnifies objects using the optical power of a single lens or a collection of lenses. A compound microscope magnifies an item considerably more thoroughly by using a series of lenses (one set expanding the picture created by another). While

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some more affordable commercial digital microscopes are straightforward single lens microscopes, compound microscopes make up the great bulk of current research microscopes. Compound microscopes may be further broken down into a number of additional sorts of microscopes, each of which has a unique optical design, a different price point, and a different intended use.

Simple microscope

A straightforward microscope gives the user an upright, enlarged virtual picture by enlarging the item by angular magnification alone. Simple magnifying tools like the magnifying glass, loupes, and eyepieces for telescopes and microscopes all make use of one or more convex lenses.

Compound microscope

A compound microscope concentrates a genuine picture of the item within the microscope by using an objective lens that is placed near to the object being studied to capture light. A second lens or combination of lenses (referred to as the eyepiece) then magnifies the picture, giving the user a larger, inverted virtual representation of the item. A compound objective and eyepiece setup enable substantially greater magnification. Exchangeable objective lenses are a common feature of compound microscopes, enabling users to swiftly alter the magnification. Additionally, a compound microscope allows for more sophisticated lighting configurations like phase contrast.

Other microscope configurations

The compound optical microscope design is available in a wide variety of specialisations. Some of these allow for customization for certain applications due to physical design differences: Often used for dissection, the stereo microscope is a low-powered microscope that offers a stereoscopic image of the material. Using a comparison microscope with two distinct light channels, two samples may be directly compared by seeing one picture in each eye. An inverted microscope is helpful for metallography or cell culture in liquid environments. Developed for inspecting connector end faces is a fibre optic connector inspection microscope. A portable microscope for analysing high-resolution sample material. Other variations of the microscope are made for various lighting methods: Petrographic microscopes are designed with polarising filters, rotating stages, and gypsum plates to make it easier to examine crystallised objects like minerals, whose optical characteristics may change depending on their orientation.Petrographic microscope-like polarising microscope. Phase-contrast illumination technique is used in phase-contrast microscopes. Epifluorescence microscopy is a kind of microscope used to examine materials containing fluorophores.

A scanning laser is employed in the confocal microscope, a popular variation of epifluorescent illumination, to light a material for fluorescence. Use of a two-photon microscope to examine fluorescence more deeply in scattering media and minimise photobleaching, particularly in samples that are still alive. Student microscopes are typically low-power microscopes with streamlined controls and perhaps subpar optics intended for classroom usage or as a first instrument for kids. Since the invention of electron microscopes, the ultramicroscope, a modified light microscope that employs light scattering to enable viewing of minuscule particles whose diameter is below or close to the wavelength of visible light (about 500 nanometers), has largely been obsolete. The tip-enhanced Raman microscope is a kind of optical microscope based on tip-enhanced Raman spectroscopy that does not have the usual resolution constraints based on

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wavelength. This microscope was created largely utilising scanning-probe platforms and all optical equipment.

Electronic microscope

An Electron microscope is a microscope with a built-in digital camera that enables computer-based sample observation. Additionally, several degrees of automation and partial computer control over a microscope are options. Greater analysis of a microscope picture is possible with digital microscopy, including quantification of a fluorescent or histological stain as well as measures of distances and regions. Commercially accessible USB microscopes with little power are also available. These effectively function as webcams with a powerful macro lens and don't often employ transillumination. The photos from the camera are shown immediately on the computer's monitor thanks to a direct USB connection. They provide inexpensive, moderate magnifications (up to roughly 200x) without the need for eyepieces. A source or sources of high-power light are often located close to the camera lens, such as an LED source.

Using sensitive photon-counting digital cameras, it is possible to perform digital microscopy under extremely low light conditions without endangering delicate biological samples. It has been shown that the danger of harm to the most light-sensitive samples may be reduced by using a light source that emits pairs of entangled photons. The sample is lighted with infrared photons in this application of ghost imaging to photon-sparse microscopy, each of which is spatially correlated with an entangled companion in the visible band allowing effective imaging by a photon-counting camera.

History

The earliest microscopes were low magnification single lens magnifying glasses since lenses in eyeglasses first were widely used in the 13th century. The first compound microscopes appeared in Europe about 1620. One was displayed in Rome in 1624, and one was given by Cornelis Drebbel in London about that time. The genuine inventor of the compound microscope has been disputed throughout the years despite several claims. One of these is a declaration made by Dutch eyeglass producer Johannes Zachariassen 35 years after it initially surfaced that Zacharias Janssen, his father, had invented the compound microscope and/or telescope as early as 1590. Johannes' testimony, which some claim is inaccurate, indicates that Zacharias would have been a little child at the time of the compound microscope's invention. If Johannes' account is true, Hans Martens, Johannes' grandfather, is said to have invented the compound microscope. It is also claimed that Janssen's competitor Hans Lippershey, who in 1608 filed a patent application for the first telescope, also invented the compound microscope. A Dutch inventor named Cornelis Drebbel, who developed the compound microscope in 1621, is one of the historians mentioned.

The compound microscope is credited to Galileo Galilei by certain sources. After 1610, he learned how to focus his telescope so that he could see little objects up close, like flies, and that he could see small objects more clearly by looking through the wrong end of the telescope. He had to stretch his 2-foot telescope out to a distance of 6 feet in order to examine objects that were so close, but that was the only drawback. Galileo saw Drebbel's compound microscope in Rome in 1624 and was inspired to build his own, more sophisticated compound microscope. In 1625, Giovanni Faber used the name "microscope" to describe the compound microscope that Galileo presented to the Accademia dei Lincei in 1624. It was known as the "small eye" or "occhiolino" by Galileo. Micron, which means little, and skopein, which means to look at, are two Greek terms that Faber combined

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to form the phrase. It's meant to be comparable to the Lincean invention that is the term "telescope." Christiaan Huygens, another Dutchman, developed a simple, achromatically corrected, two-lens ocular system in the late 17th century, which was a crucial step in the creation of the microscope. Despite being produced today, the Huygens ocular has a small field of vision and a few other minor flaws.

A painting of bees by Francesco Stelluti from 1630 is the earliest image known to have been published using a microscope. Antonie van Leeuwenhoek (1632–1724) is credited for bringing the microscope to biologists, despite the fact that simple magnifying lenses had previously been developed in the 16th century. With a single, powerful, but small lens, Van Leeuwenhoek developed his own simple microscopes. They were difficult to operate, but they allowed Van Leeuwenhoek to view exact images. For the compound microscope to be able to generate pictures of the same quality as van Leeuwenhoek's basic microscopes, about 150 years of optical study were required due to the difficulties in building several lenses. One of the earliest and biggest microscopic investigations of cholera in the United States was carried out in the 1850s by John Leonard Riddell, a professor of chemistry at Tulane University, who also invented the first working binocular microscope at the same time. Even though the basics of microscope technology and optics have been around for more than 400 years The techniques used in sample lighting to create the high-quality images we see today are much more modern.

Köhler illumination was developed in August 1893 by August Köhler. This method for sample illumination overcomes many of the problems with older techniques and creates highly even lighting. Before the invention of Köhler illumination, it was always possible to make out the image of the light source, such as a filament from a lightbulb. The invention of phase contrast lighting, which enables imaging of translucent objects, won Dutch researcher Frits Zernike the 1953 Nobel Prize in Physics. Extremely transparent materials, including living human cells, may be seen without the need of staining methods by exploiting interference rather than absorption of light. The theory for differential interference contrast microscopy, another interference-based imaging method, was presented by Georges Nomarski only two years later, in 1955.

Fluorescence microscope

When used in a fluorescence microscope, fluorescent chromophores known as fluorochromes may absorb light energy from a light source and then release it as visible light. Both fluorescent stains that are applied to the material to produce contrast and naturally luminous compounds (like chlorophylls) are considered fluorochromes. Fluorochromes include dyes like Texas red and FITC. The nucleic acid dyes acridine orange and 4',6'-diamidino-2-phenylindole (DAPI) are more examples. The chromophores in the specimen absorb the excitation light and produce longer-wavelength visible light when it is transmitted through the microscope, which is often a kind of EMR with a short wavelength, such ultraviolet or blue light. In order for only visible light to enter through the ocular lens, the excitation light is then filtered away (partially because UV radiation is hazardous to the eyes). This results in a dark backdrop and vibrant images of the specimen.

Clinical microbiology makes extensive use of fluorescent microscopes. They may be used to locate specific chemicals and cellular structures inside a cell, identify diseases, locate certain species within an environment, or locate pathogens. Additionally, methods for separating live from dead cells using fluorescence microscopy have been developed based on how well they absorb various fluorochromes. To highlight various structures or characteristics, several fluorochromes may sometimes be employed on the same material. Immunofluorescence, one of the most significant

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uses of fluorescence microscopy, is a method for identifying specific pathogens by determining whether antibodies attach to them. (Antibodies are protein molecules created by the immune system that cling to certain diseases to destroy or suppress them.) There are two methods for implementing this technique: the direct immunofluorescence assay (DFA) and the indirect immunofluorescence test (IFA). Particular antibodies, such as those that target the rabies virus, are stained with a fluorochrome in DFA. The antibodies' binding to the pathogen may be seen under a fluorescent microscope if the specimen contains the targeted pathogen. Due of the stained antibodies' ability to bind to the pathogen directly, this is known as a primary antibody stain.

Instead of using primary antibodies, IFA uses secondary antibodies that are stained with a fluorochrome. Although they bind to main antibodies, secondary antibodies do not directly adhere to the pathogen. The fluorescent secondary antibodies may be seen attaching to the primary antibodies as they bind to the pathogen with the unstained primary antibodies. The creation of fluorescent probes for particular cell structures is a critical component of contemporary biological imaging. Fluorescence microscopy uses a limited range of light wavelengths to illuminate the material via the objective lens, in contrast to conventional transilluminated light microscopy. Fluorophores in the sample react to this light, and the sample subsequently emits light with a larger wavelength. The picture is made up of this light that was released. Chemical fluorescent stains, including the DNA-binding DAPI, have been employed to mark certain cell structures since the middle of the 20th century. The use of fluorescently labelled antibodies to identify particular proteins in a sample is a more recent discovery. Another is the use of fluorescent proteins like GFP, which living cells may produce to make them fluorescent. Components The fundamental elements of the light path are shared by all contemporary optical microscopes intended for transmitted light viewing of samples. The great majority of microscopes also have the same "structural" parts:

A specimen is the thing that is being examined. A glass slide with the specimen is put on it, and the glass slide is then fastened to the microscope's stage (or platform). Using the x-y mechanical stage knobs, the specimen on the slide is then positioned above the light after the slide has been fastened. These knobs only change the stage's surface slide; the stage itself cannot be raised or lowered. To concentrate the picture after the specimen is centred over the light, the stage position may be increased or lowered. While using 4 and 10 objective lenses, the coarse focusing knob is used for significant movements; when using 40 or 100 objective lenses, the fine focusing knob is utilised for little movements. Since there is less light per unit area of the picture when it is enlarged, the image becomes darker. Therefore, powerful illumination is needed to generate the highly magnified pictures produced by microscopes. This illumination in a brightfield microscope comes from an illuminator, which is normally a high-intensity bulb underneath the stage. Condenser lens (placed below the stage) directs light from the illuminator upward, where it is focused to illuminate the specimen as effectively as possible. The connected condenser focus knob may be used to change the condenser's location; however, once the ideal distance has been found, the condenser shouldn't be moved to alter the brightness. If less-than-maximum light levels are required, it is simple to modify the quantity of light reaching the specimen by opening or closing a diaphragm situated between the condenser and the object. The rheostat, a dimmer switch that regulates the brightness of the illuminator, may sometimes also be used to change brightness.

An image is produced by a brightfield microscope by shining light from the illuminator at the specimen, which is then diffracted, absorbed, reflected, or refracted differently by various structures. In certain areas of the specimen, chromophores—pigments that absorb and reflect

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specific wavelengths of light—can cause distinct hues to behave in different ways. Stains are often used to artificially add chromophores to the material, improving contrast and resolution. Structures in the specimen will typically look darker, to varying degrees, than the light backdrop, producing the sharpest pictures at magnifications up to around 1000 times. Even further magnification would just result in a bigger picture with worse resolution. As a result, we can detect items as tiny as bacteria, which can be seen at 400 nm or thereabouts, but not smaller ones like viruses.

When light travels through the thin layer of air that is present between the specimen and the lens at extremely high magnifications, resolution may be hampered. This is because air scatters light rays before they can be focussed by the lens since air and glass have very different refractive indices. This issue may be fixed by placing a drop of oil between the specimen and an oil immersion lens, a unique lens made specifically to be used with immersion oils. The oil increases the greatest angle at which light exiting the specimen may reach the lens since it has a refractive index that is extremely close to that of glass. As a result, the amount of light captured and the picture resolution both rise. For various forms of illumination, a variety of oils may be employed. The eyepiece, also known as the ocular lens, is a cylinder that houses two or more lenses and serves to focus the picture for the eye. The upper end of the body tube is where the eyepiece is placed. Since eyepieces may be switched out, a wide variety of eyepieces with various magnification levels can be used. Eyepieces typically have magnifications of 5, 10, 15, and 20 times normal. The objective lens and eyepiece of certain high-performance microscopes are matched optically to provide the highest optical performance. With apochromatic goals, this happens most often.

One or more objective lenses that gather light from the sample are located at the bottom end of a conventional compound optical microscope. Typically, a glass single- or multi-element compound lens is housed within a cylindrical housing that houses the objective. Usually, a circular nose piece with three objective lenses fitted into it may be turned to choose the correct objective lens. These configurations are intended to be parfocal, which implies that the sample maintains focus while using a microscope with different lenses. Magnification and numerical aperture are the two factors that define microscope objectives. The latter normally varies from 0.14 to 0.7, corresponding to focal lengths of around 40 to 2 mm, whereas the former typically ranges from 5 to 100. Greater numerical apertures and shallower depths of focus are often characteristics of objective lenses with higher magnifications. Some high-performance objective lenses may need matching eyepieces to provide the optimal optical performance.

Oil Immersion

To achieve better resolution at high magnification, some microscopes utilise water- or oil-immersion objectives. These are used in conjunction with an index-matching medium, such as immersion oil or water, and a matched cover slip placed between the objective lens and the sample. The objective lens may have a numerical aperture greater than 1 because the index-matching material has a higher refractive index than air, which enables the transmission of light from the specimen to the objective lens' outer face with the least amount of refraction. One may get numerical apertures of up to 1.6. A more significant numerical aperture enables the capture of more light, enabling close examination of minute details. Typically, an oil immersion lens magnifies between 40 and 100 times.

Focus Knobs

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With independent adjustments for coarse and fine focusing, adjustment knobs raise and lower the stage. The microscope's settings allow it to adapt to specimens of various thicknesses. Older models of microscopes had a fixed stage and the focus adjustment wheels moved the microscope tube up and down in relation to the stand.

Frame

In order to provide the requisite stiffness, the whole optical system is often mounted to a rigid arm, which is then coupled to a sturdy U-shaped foot. The viewing angle may be modified due to an adjustable arm angle. Various microscope controls may be mounted on the frame. This often has focusing controls, typically a big knurled wheel to adjust coarse focus and a smaller knurled wheel to regulate fine focus. Controls for the condenser's adjustment and/or light controls are examples of additional functionality.

Stage

The stage is a platform that is located underneath the objective lens and holds the specimen being examined. There is a hole in the stage's middle through which light illuminates the specimen. The stage often features arms for holding slides, which are rectangular glass plates with a specimen placed on them and typically measuring 25 mm by 75 mm in size. Moving a slide by hand is impractical with magnifications greater than 100. A mechanical stage, which is characteristic with microscopes with medium and higher price tags, enables minute adjustments of the slide through control knobs that reposition the sample/slide as needed. If a microscope did not come with a mechanical stage, one could be able to be added. For emphasis, every stage moves up and down. Slides on a mechanical stage move along two horizontal axes to position the specimen for close examination of the specimen's features. To help the user centre the specimen on the stage, focusing begins at a lower magnification. When switching to a higher magnification, the stage must be raised vertically to allow for refocusing at the higher magnification. A little adjustment to the horizontal specimen position may also be necessary. The mechanical stage's purpose is to allow for horizontal specimen position modifications. For toddlers, it is advisable to start with prepared slides that are centred and focus readily regardless of the focus setting chosen due to the difficulties in preparing specimens and mounting them on slides.

Source of Light

Light may come from a variety of sources. Light is directed via a mirror in its most basic form. Although lighting utilising LEDs and lasers is becoming prevalent, the majority of microscopes have their own adjustable and controlled light source, often a halogen bulb. More costly equipment often have Köhler lighting.

Condenser

Using a lens called a condenser, the illumination source's light may be focused on the sample. To control the illumination's quality and intensity, the condenser could additionally include additional components like a diaphragm and/or filters. Additional optical components need to be properly oriented in the light path for illumination methods including differential interference contrast, phase contrast, and dark field microscopy.

Magnification

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The sum of the eyepiece and objective lens powers determines the actual power or magnification of a compound optical microscope. For instance, a total magnification of 1,000 times is produced by a 100 times objective lens magnification plus a 10 times eyepiece magnification. At magnifications more than 1,000x, resolved features are possible in altered surroundings such those created by the use of oil or UV light.

Techniques for illumination

It is possible to produce a picture with increased contrast from a sample using a variety of strategies that alter the light path. Cross-polarized light, dark field, phase contrast, and differential interference contrast lighting are some of the main methods for enhancing contrast from the sample. Cross-polarized light and particular contrast-enhanced slides are used in a new method (Sarfus) for the observation of nanometric samples. A piece of tissue paper was illuminated using four different transilumination procedures to create contrast. 1.559 m/pix. There are several methods that may be utilised to retrieve additional types of data from samples using modern microscopes, allowing for more analysis than simply seeing the transmitted light picture. A standard compound microscope isn't enough for the majority of these tasks. An optical microscope picture of cells in a medical smear test at a 40x magnification using the wet mount method, which involves mounting the specimen on a glass slide and combining it with a salt solution.

In microelectronics, nanophysics, biotechnology, pharmaceutical research, mineralogy, and microbiology optical microscopy is widely employed. When dealing with tissues, the area is known as histology. Optical microscopy is also utilised in smear tests on free cells or tissue pieces. Binocular microscopes are often used in industrial settings. The use of dual eyepieces lessens eye fatigue brought on by extended workdays at a microscope station, in addition to applications requiring real depth perception. Long-focus or long-working-distance microscopes are useful in several situations. An thing could need to be inspected via a window, or the goal might be jeopardised by industrial topics. Such optics are comparable to telescopes with a close-focus feature.

Precision measurements are made with the use of measuring microscopes. There are two fundamental categories. To enable measuring distances in the focal plane, one uses a graded reticle. The other, more traditional model contains basic crosshairs and a micrometre mechanism for moving the object in relation to the microscope. Some situations where a laboratory microscope would be cumbersome have found use for very tiny, portable microscopes.

Limitations

The diffraction cap carved into a memorial honouring Ernst Abbe. Point objects appear as fuzzy discs encircled by diffraction rings at very high magnifications with transmitted light. Airy discs are what they are. The capacity to discriminate between two closely spaced Airy discs is considered a measure of a microscope's resolving power (or, in other words the ability of the microscope to reveal adjacent structural detail as distinct and separate). The resolution of tiny details is constrained by these effects of diffraction. The numerical aperture (NA), refractive material used to make the objective lens, and wavelength of the light () all have an impact on the size and intensity of the diffraction patterns. Therefore, there is a finite limit, known as the diffraction limit, beyond which it is difficult to discern individual points in the objective field. The resolution d may be expressed as follows, assuming that optical aberrations are minimal across the whole optical setup: green light, with a wavelength of 550 nm, is often assumed. The maximum practicable NA

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with air as the external medium is 0.95, while with oil, it may reach 1.5. With conventional lenses, the lowest value of d that can be achieved in practise is about 200 nm. The resolution was improved to below 100 nm using a novel lens design that makes use of multiple light scattering.

There are several methods for increasing resolution over the previously mentioned limit of transmitted light. Although resolution was constrained in their experimental research, Courjon and Bulabois's 1979 description of holographic methods is also capable of surpassing this resolution threshold. More approaches may be used with fluorescent samples. Vertico SMI, stimulated emission depletion, and near field scanning optical microscopy are a few examples. A microscope that can identify a single molecule was described as a teaching aid in 2005. The methods for going above the diffraction limit continue to be specialised and constrained, despite tremendous advancement in the previous ten years. While most approaches strive to improve lateral resolution, several techniques also aim to enable examination of very thin samples. For instance, Sarfus techniques lay the tiny sample on a surface that improves contrast, allowing one to directly see films as thin as 0.3 nanometers. The invention of super-resolved fluorescence microscopy earned Stefan Hell, William Moerner, and Eric Betzig the 2014 Nobel Prize in Chemistry.

A light optical technique of the so-called point spread function (PSF) engineering is termed spatially modulated illumination microscopy (SMI). In order to increase optical resolution, increase the precision of distance measurements of fluorescent objects that are small relative to the illumination light's wavelength, or extract additional structural parameters in the nanometer range, these processes modify a microscope's PSF in a suitable way. Microscopy for localization SPDM (spectral precision distance microscopy) SPDM (spectral precision distance microscopy), the fundamental localization microscopy technology, is a light optical process of fluorescence microscopy that enables position, distance, and angle measurements on "optically isolated" particles (for example, molecules), well below the theoretical limit of resolution for light microscopy. When a particle or molecule is said to be "optically isolated," it indicates that at any one moment, only one instance of it is being detected in an area that is the size specified by standard optical resolution (usually between 200 and 250 nm in diameter). Because each molecule in this area has a unique spectral tag, this is achievable (e.g. different colours or other usable differences in the light emission of different particles).

If certain photo-physical parameters are met, localization microscopy may be performed using a variety of common fluorescent dyes, including GFP, Alexa dyes, Atto dyes, Cy2/Cy3 dyes, and fluorescein molecules. A single laser wavelength of a sufficient intensity is adequate for nanoimaging when using this so-called SPDM (physically modifiable fluorophores) technology. Combining structured illumination SMI with localization microscopy for conventional fluorescent dyes SPDMphymod allows for 3D super resolution microscopy with fluorescent dyes. Although stimulated emission depletion is a straightforward illustration of how better resolution may transcend the diffraction limit, it has significant drawbacks. STED is a fluorescence microscopy method that use a series of light pulses to cause a tiny subpopulation of fluorescent molecules in a sample to glow. The centre of each molecule-produced diffraction-limited point of light in the picture corresponds to the position of the molecule. Since there aren't many fluorescing molecules, the patches of light aren't likely to overlap, making precise placement possible. Then, in order to create the picture, this procedure is done several times. In 2006, Stefan Hell of the Max Planck Institute for Biophysical Chemistry won the 10th German Future Prize, and in 2014, he received the Nobel Prize in Chemistry for his work developing the STED microscope and related techniques.

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Since electrons and X-rays have shorter wavelengths than light and hence have lower diffraction limits, they may be used to achieve considerably greater resolutions. The atomic beam imaging system (also known as the atomic nanoscope) has been put out and extensively debated in the literature as a means of making the short-wavelength probe non-destructive, however it is not yet cost-effective compared to traditional imaging systems. STM and AFM are scanning probe methods that move a tiny probe over the surface of the material. The size of the probe in these situations determines the resolution; micromachining methods may create probes with tip radii of 5–10 nm. Furthermore, techniques like electron or X-ray microscopy need a vacuum or partial vacuum, which restricts their use to living and biological material (with the exception of an environmental scanning electron microscope). Sample size is further constrained by the specimen chambers required for all such equipment, and sample manipulation is more challenging. These techniques result in photos that lack some information since colour cannot be seen. However, they are crucial for examining molecular or atomic effects, such age hardening in aluminium alloys or the microstructure of polymers.

Non-optical Microscopy

The following are a few instances of non-optical microscopy:

SEM, or scanning electron microscopy, is a technique that involves passing high-energy electrons across a sample while simultaneously releasing and recording many emissions. This kind of microscopy allows for magnification of five to 500,000 times. In a scanning probe microscope, highly pointed probes are used to pass over the specimen's surface and interact with it rather than light or electrons. This generates data that can be combined to create pictures that can be magnified up to 100,000,000 times. One may view individual atoms on surfaces at such high magnifications. These methods have mostly been used in research as opposed to diagnosis up to now.

There are two different kinds of scanning probe microscopes: the atomic force microscopy (AFM) and the scanning tunnelling microscope (STM). A probe is run over the specimen in an STM in order to produce the potential for an electric current to flow between the probe and the specimen. The strength of the current depends on how close the probe is to the specimen and how many electrons are quantum tunnelling between them for this current to occur. The probe is positioned horizontally above the surface, and the current's intensity is gauged. In a resolution that allows for the detection of individual atoms, scanning tunnelling microscopy is useful at mapping the structure of surfaces.

AFMs feature a narrow probe that is passed just above the specimen, similar to an STM. An AFM, on the other hand, creates a constant current and detects fluctuations in the height of the probe tip as it passes over the specimen rather than monitoring current variation at a fixed height above the specimen. Van der Waals, capillary, chemical bonding, electrostatic, and other forces between the atoms cause the probe tip to move up and down as it passes over the sample. Utilizing Hooke's law of elasticity, the probe tip's deflection is calculated and quantified, and this data is then utilised to create photographs of the specimen's surface with atomic-level resolution. The method of transmission electron microscopy captures electrons as they pass through a thin substrate. A 50,000,000x magnification is possible with this sort of equipment. They can provide very accurate three-dimensional images of a material, allowing for investigation of its topography, structure, and composition. This kind of microscopy may be used for both biological and industrial purposes, and it can also be used to detect impurities or flaws in small objects like microelectronics.

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The only difference between optical and transmission electron microscopy equipment is that high-energy electrons are employed instead of light in transmission electron microscopy. They are used in a wide range of fields, including as metallurgy, forensics, medical, and biological research. Scanning probe microscopy allows for analysis at the nanoscale, down to the level of a single atom (SPM). To detect deflections, the device consists of a cantilever with an atom-sized sharp tip that is moved across a surface. These deflections are used to produce an image by reflecting a laser off the top of the cantilever and capturing the results.

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CHAPTER 2

Sterilization and Disinfection

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How clean is clean? Most people would not want to eat from surfaces that they wash their automobiles and clean. In a similar vein, although we may use dishwasher-cleaned cutlery for eating, we cannot use the same dishwasher to clean surgical tools. These instances show that the word "clean" is a relative one. Vacuuming, cleaning dishes, and car washing all lessen the microbial load on the treated goods, making them seem "cleaner." However, their intended usage will determine if they are "clean enough." These goods don't need to be as clean as silverware since people don't often eat off of automobiles or rugs. Similarly, since silverware is not used in invasive surgery, it does not need to be as clean as surgical equipment, which has to be sterilised in order to avoid infection. Why not take precautions and sanitise everything? It is not only harmful, but also impracticable, to sterilise everything we come into touch with. As this chapter will show, sterilisation methods often call for lengthy, labor-intensive processes that might harm users or decrease the quality of the object being sterilised. To guarantee that an item is "clean enough," the user must take the planned usage into account while selecting a cleaning procedure. The term "sterilization" refers to the process of eliminating all live germs, including bacterial spores. Physical, chemical, and physiochemical methods may all be used to sterilise anything. Chemisterilants are substances that are used to sterilise objects.

Disinfection is the process of getting rid of the majority of harmful germs on inanimate items, with the exception of bacterial spores. It is possible to disinfect via chemical or physical means. Disinfectants are substances that are utilised in cleaning. Not all disinfectants can completely eradicate all bacteria since they have varied ranges of action. Filtration is one kind of disinfection that separates out the bacteria instead of killing them. Disinfection is not a need, although sterilization is. They are not interchangeable.

Decontamination is the elimination of potentially harmful pathogenic bacteria from objects using a sterilization or disinfection procedure. It is the process of removing, incapacitating, or destroying live organisms on a surface such that they are no longer contagious via physical or chemical techniques. In public health systems, sanitization refers to the process of chemical or mechanical cleaning. Typically used by the food sector. It lowers the number of bacteria on dining utensils to safe, livable levels. The use of methods (such as using gloves, air filters, UV rays, etc.) to create a microbe-free environment is known as asepsis. Using chemicals (antiseptics) to rid skin or mucous membranes of pathogenic microorganisms is known as antisepsis. Bacteriostasis is a state in which the bacteria are prevented from multiplying but are not killed. The substance that can eliminate or inactivate bacteria is known as a bactericidal. Depending on their range of activity, these chemicals may go by different names, such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal, or germicidal.

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Antibiotics are compounds made by one type of microbe that prevent or eradicate another microbe. Frequently, the phrase is used in a broader sense to refer to both synthetic and semi-synthetic antimicrobial agents. Sterilization is the process of purging materials of infectious organisms. To sterilise items and preserve them for a long time, this is done. When something is not sterilised, the user may become ill. Therefore, it shouldn't be taken for granted. There are many sterilising methods, including:

The following factors can affect heat sterilisation:

1) The effectiveness of moist heat is greater than that of dry heat.
2) Temperature and time: These two variables have an inverse relationship. The time required decreases with rising temperature.
3) Microorganism count: The more microorganisms present, the higher the temperature or the longer the time period needed.
4) Microorganism nature: Heat sensitivity varies depending on species and strain of microorganism. Spores have a high tolerance for heat.
5) Material: Highly contaminated items need to be exposed for a longer period of time or at a higher temperature. Sterilization at lower temperatures is required for some heat-sensitive items.
6) Organic material is present: The time needed is increased by organic materials like protein, sugars, oils, and fats.

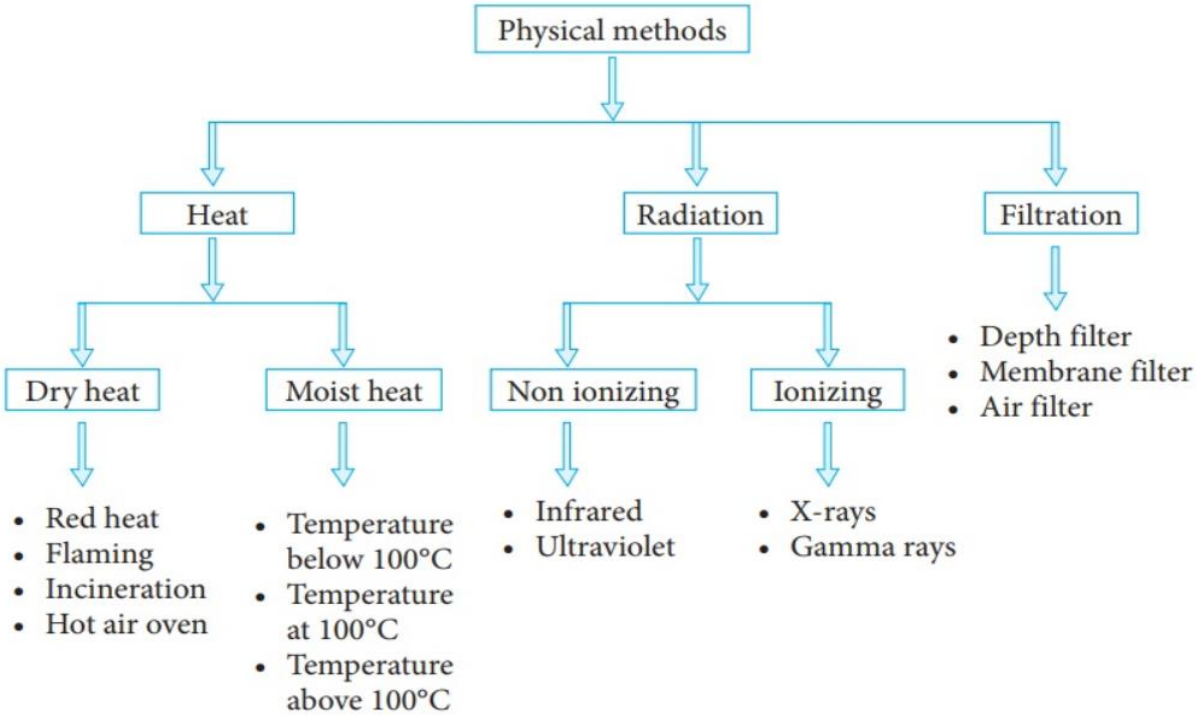

Figure 2.1: Physical methods of sterilisation

Heat's effects include protein denaturation, oxidative damage, and toxic effects from excessive electrolyte levels. The moist heat causes the proteins to coagulate and denature. In practice, moist heat is more effective than dry heat. Dry heat requires a higher temperature than moist heat in order

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to kill microbes. The shortest amount of time needed for a suspension of organisms to die at a predetermined temperature in a particular environment is known as the thermal death time.

Wet heat

Red heat: By holding items in a Bunsen flame until they are red hot, such as bacteriological loops, straight wires, forceps tips, and searing spatulas, they can be sterilised. This is an easy method for sterilising such items, but it can only be used on items that can be heated to redness in flame.

Flaming: This technique involves passing the object through a Bunsen flame without heating it to a reddish colour. Scalpels, test tube mouths, flasks, glass slides, and cover slips are among the items that are briefly heated in the flame. Although the majority of vegetative cells are destroyed, there is no assurance that spores would also perish after such a brief exposure. The items that can be used with this method are also those that can be exposed to flame. It's possible for the glassware to crack.

Incineration: Using an incinerator, this technique burns contaminated materials to ash. Items like soiled bandages, animal carcasses, pathological material, and bedding, among others, should be burned. This method results in the loss of the object, making it only appropriate for objects that need to be disposed of. Polystyrene materials shouldn't be burned because doing so produces thick smoke. Louis Pasteur introduced the hot air oven technique. In an electrically heated oven, items that need to be sterilised are exposed to a high temperature (160o C) for one hour. A fan is used to evenly distribute heat throughout the chamber because air is a poor conductor of heat. By means of radiation, conduction, and convection, the heat is transferred to the object. The oven needs to have sufficient insulation, a thermostat control, temperature indicators, and mesh shelves. Metal objects (such as forceps, scalpels, and scissors), glass objects (such as petri dishes, pipettes, flasks, and all-glass syringes), swabs, oils, grease, petroleum jelly, and some pharmaceutical products are among the items that are sterilised.

Sterilization procedure: To prevent breakage, the items to be sterilised must be completely dry before being placed inside. It is important to space items far enough apart to allow for free airflow between them. Cotton wool plugs must be placed inside test tubes, flask mouths, and pipette ends. Metal canisters can be used to arrange items like pipettes and petri dishes before being filled. Glass items must be individually wrapped in aluminium foil or kraft paper.

Sterilization cycle: This accounts for the time it takes for the items to reach the sterilising temperature, maintain that temperature for a predetermined amount of time (holding time), and then cool down. For holding time, there are three different temperature-time relationships: 60 minutes at 160°C, 40 minutes at 170°C, and 20 minutes at 180°C. A 10 degree increase in temperature cuts the sterilising time in half. To avoid glassware breaking, the hot air oven must not be opened until the interior temperature has dropped below 60°C.

Controlling sterilisation: There are three ways to evaluate the effectiveness of the sterilisation process: physical, chemical, and biological.

1) Physical: A thermocouple and a temperature chart recorder.
2) Chemical: No. 3 Browne's tube (green spot, colour changes from red to green)

Heat Sterilization: In a hot air oven, 106 spores of *Bacillus subtilis* var. *niger* or *Clostridium tetani* on paper strips are placed inside envelopes. The strips are removed after the sterilisation

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cycle is finished, inoculated into thioglycollate broth or cooked meat medium, and then incubated for 3-5 days at 37°C. The spores should be killed by proper sterilisation, and growth should not occur.

Benefits: It is a reliable technique for sterilising heat-resistant items. Even after sterilisation, the items are still dry. Oils and powders can only be sterilised using this technique.

Cons: Hot air has poor penetration because air is a poor conductor of heat. Cotton wool and paper may lightly char. Glasses may smoke. It requires more time than an autoclave.

Infrared radiation: By producing heat, infrared radiation sterilises objects. Sterilization items are placed on a conveyer belt that moves through a tunnel that is heated by infrared radiators to 180°C. The items are in contact with that temperature for 7.5 minutes. Sterilized items included glassware and metallic objects. The central sterile supply department uses it the most. Since it needs specialised equipment, a diagnostic laboratory cannot use it. Utilizing Browne's tube No. 4, efficiency can be evaluated (blue spot).

Moist heat

Moist heat causes the proteins to coagulate and denature. At temperatures lower than 100 ^{o}C. Louis Pasteur first used this method, called pasteurisation. This process is currently used in the dairy and food industries. Pasteurization can be done using either the holder method, which is heated at 63 °C for 30 minutes, or the flash method, which is heated at 72 °C for 15 seconds before being quickly cooled to 13 °C. Ultra-High Temperature (UHT), 140°C for 15 sec., and 149°C for 0.5 sec. are additional pasteurisation techniques. The majority of milk-borne pathogens, such as Salmonella, Mycobacteria, Streptococci, Staphylococci, and Brucella, can be eliminated using this method; however, Coxiella may survive pasteurisation. Phosphatase and methylene blue tests are used to determine effectiveness.

Vaccine bath: By heating in a water bath at 60 °C for one hour, the contaminating bacteria in a vaccine preparation can be rendered inactive. Spores survive, and only vegetative bacteria are destroyed.

Serum bath: Heating in a water bath at 56 °C for one hour on several consecutive days will inactivate the contaminating bacteria in a serum preparation. Higher temperatures will cause the serum's proteins to coagulate. Spores survive, and only vegetative bacteria are destroyed. This process of "inspissation" solidifies and sanitises media containing eggs and serum. On three separate days, the medium containing serum or eggs is placed on the slopes of an inspissator and heated at 80–85 °C for 30 minutes. The vegetative bacteria would die on the first day, and the spores that would germinate on the second day would be killed on the third day. In between inspissation’s, spore germination is necessary for the process to occur. This method cannot be regarded as sterilisation if the spores do not germinate.

Boiling water kills the majority of vegetative bacteria and viruses instantly at a temperature of 100oC. Staphylococcal enterotoxin is one bacterial toxin that can withstand heat. Boiling does not replace sterilisation because some bacterial spores are resistant to it and can survive. 2% sodium bicarbonate can be added to increase the killing activity. Certain metal objects and glassware can be disinfected by boiling water for 10 to 20 minutes when absolute sterility is not required. During this time, the boiler's lid must not be opened.

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Steam at 100°C: The articles are exposed to free steam at 100°C rather than being kept in boiling water. Arnold's and Koch's steamers have typically been used. The same function can be accomplished with an autoclave (with the discharge tap open). A steamer is a metal cabinet with a conical lid and perforated trays to hold the items. Water is heated and poured into the bottom of the steamer. When exposed to the generated steam for 90 minutes, the items are sterilised. Steam sterilises media like TCBS, DCA, and selenite broth. Since sugar and gelatin in a medium may break down during an autoclave, they are subjected to free steaming for 20 minutes over the course of three days. After John Tyndall, this procedure is referred to as tyndallization, fractional sterilisation, or intermittent sterilisation. The first exposure kills the vegetative bacteria, and subsequent exposures kill the spores that germinate the following day. The spores' ability to germinate is essential to the process' success:

Autoclave: Using an autoclave, sterilisation can be accomplished successfully at temperatures higher than 100oC. At atmospheric pressure, water boils at 100°C; however, as pressure is raised, the temperature at which the water boils also rise. In an autoclave, water is brought to a boil inside a sealed chamber. The boiling point of water increases along with pressure. The temperature inside the autoclave is reported to be 121oC at a pressure of 15 lbs. Items are sterilised after 15 minutes at this temperature. Higher temperatures or longer times are used to destroy the infectious agents linked to spongiform encephalopathies (prions); 135°C or 121°C for at least an hour are recommended.

Steam has several benefits, including the ability to penetrate deeper than dry air, the ability to moisten spores (which are necessary for the coagulation of proteins), the ability to release latent heat when steam condenses on a cooler surface, and the ability to draw in fresh steam.

Kinds of autoclaves

Laboratory autoclaves that are straightforward and "pressure-cooker type," steam jacketed downward displacement autoclaves, and high-pressure pre-vacuum autoclaves are all available.

Figure 2.2: Types of Autoclave

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A basic autoclave has a horizontal or vertical cylindrical body with a heating element, a perforated tray to hold the objects, a screw-clamp-affixed lid, a pressure gauge, a safety valve, and a discharge tap. The items that need to be sterilised cannot be packed tightly. The cotton plugs and screw caps need to fit loosely. The water is heated even though the lid is closed and the discharge tap is left open. The steam pushes air out of the discharge tap as the water begins to boil. The discharge tap is closed once all the air has been expelled and steam has begun to emerge. Inside, the pressure is permitted to increase by up to 15 lbs per square inch. After holding the items under this pressure for 15 minutes, the heating is turned off and the autoclave is left to cool. The discharge tap is opened to let air in once the pressure gauge indicates that the pressure is equal to atmospheric pressure. The lid is then opened, and items are taken out. Sterilized items include dressings, culture media, some equipment, linen, etc. Articles should not be tightly packed, the autoclave should not be overloaded, air discharge should be complete and there shouldn't be any trapped air inside, caps should not be too tight on bottles and flasks, the autoclave should not be opened until the pressure has decreased or the contents will boil over, articles should be wrapped in paper to prevent drenching, and bottles shouldn't be filled to the brim.

Advantages

Faster than a hot air oven and very effective for sterilisation.

Drawbacks

Drenching and wetting of items may occur, trapped air may reduce effectiveness, and cooling takes a long time.

Controlling Sterilisation

Thermocouples, temperature chart recorders, and automatic process control are examples of physical methods. Browne's tube No. 1 (black spot), succinic acid, which has a melting point of 121 ^{o}C, and Bowie Dick tape are all components of the chemical process. On items going into the autoclave, Bowie Dick tape is used. The tape will show dark brown stripes if the procedure was successful. The biological method uses a paper strip that has 10^{6} *Geobacillus stearothermophilus* spores on it.

Radiation

Ionizing and non-ionizing radiation of different types are both used. Ionizing rays have good penetrating power while non-ionizing rays have low energy and poor penetrating power. Radiation is sometimes referred to as "cold sterilisation" because it doesn't produce heat. Fruits and vegetables are irradiated to extend their shelf life by up to 500 % in some regions of Europe.

Ionising Radiation

Non-ionizing radiation has a wavelength longer than visible light. UV rays have a microbicidal wavelength that ranges from 200 to 280 nm, with 260 nm being the most effective. Using a high-pressure mercury vapour lamp, UV rays are produced. The microorganisms' maximum level of absorption occurs at this wavelength, which causes the germicidal effect. Thymine-thymine dimers are created by UV rays, which ultimately prevent DNA replication. When cells receive non-lethal doses of UV radiation, mutations are easily induced. When exposed to the effective UV radiation, microorganisms like bacteria, viruses, yeast, etc. are rendered inactive in a matter of seconds. UV rays are viewed as useful in surface disinfection because they don't kill spores. Hospital wards,

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operating rooms, virus laboratories, corridors, etc. are all cleaned with UV rays. The use of UV rays has drawbacks such as low penetration, short bulb life, some bacteria have DNA repair enzymes that can repair UV damage, organic matter and dust block the rays' path, and the rays are harmful to skin and eyes. Glass, paper, and plastic are not penetrated by it.

Ionizing rays come in two varieties: electromagnetic and particulate rays.

Gamma rays are electromagnetic while electron beams are particulate in nature. A linear accelerator generates high-speed electrons from a heated cathode. Sterilization of items like syringes, gloves, dressing packs, food, and pharmaceuticals is accomplished using electron beams. Sterilization takes only a brief amount of time. The instruments, in contrast to electromagnetic rays, can be turned off. Poor penetrating power and the need for expensive equipment are disadvantages. Some radioactive isotopes undergo nuclear disintegration, which emits electromagnetic rays like gamma rays (Co60, Cs137). Compared to electron beams, they are more incisive but require a longer exposure time. The nucleic acid of the microorganism is damaged by these high-energy radiations. At 2.5 megarads, all bacteria, fungi, viruses, and spores are destroyed. Commercially, it is used to sterilise plastic syringes, disposable petri dishes, antibiotics, vitamins, hormones, glassware, and fabrics. A few drawbacks are that, unlike electron beams, they cannot be turned off, that glassware tends to turn brown, and that fabrics lose some of their tensile strength. Certain foods lose flavour when exposed to gamma radiation. To assess the sterilisation process, *Bacillus pumilus* E601 is used.

Filtration

Microbes are not killed by filtration; rather, it separates them. When a solution cannot be autoclaved, membrane filters with pore sizes between 0.2-0.45 m are frequently used to filter out the particles. In liquids that are heat labile, such as serum, antibiotic solutions, sugar solutions, and urea solution, it is used to get rid of microbes. Filtration has many uses, including removing bacteria from culture media ingredients, making bacteria-free virus and phage suspensions, determining virus sizes, separating toxins from culture filtrates, counting bacteria, clarifying fluids, and purifying hydatid fluid. Using vacuum pumps to create positive or negative pressure helps with filtration. Depth filters are the more traditional filters made of earthenware or asbestos.

Various types of filters include

1. **Earthenware filters:** These filters are constructed of porcelain or diatomaceous earth. Typically, they are baked into the form of a candle. The following are some types of earthenware filters:

    a. Pasteur-Chamberland filter: Made of porcelain, these candle filters are imported from France (sand and kaolin). Doulton is a similar filter made in Britain. The porosities used to create Chamberland filters are categorised as L1, L1a, L2, L3, L5, L7, L9, and L11. Filters from Doulton are P2, P5, and P11.
    b. Kieselguhr, a fossilised diatomaceous earth mineral found in Germany, is used to make Berkefeld filters. According to their porosity (pore size), they are available in three grades: V (veil), N (normal), and W. (wenig). Use of *Serrtia marcescens* culture suspension (0.75 m) to test the V grade filter's quality.
    c. The Mandler filter is an American product made of plaster of Paris, asbestos, and kieselguhr.

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2. Asbestos filters: Chrysotile asbestos, which is chemically composed of magnesium silicate, is used to make these filters. They are compressed to create discs that can only be used once. The metal mount that the disc is housed in has undergone autoclave sterilisation. There are three grades available: HP/PYR (for pyrogen removal), HP/EKS (for absolute sterility), and HP/EK (for clarifying).

3. Sintered glass filters: These are created from glass that has been finely ground and sufficiently fused to cause small particles to stick together. Typically, they come in the shape of a glass funnel with a disc fused to it. The average pore size of Grade 5 filters is between 1-1.5 m. They are cleaned with warm concentrated H2SO4 and autoclaved after being rinsed under running water in the opposite direction.

4. Membrane filters: These filters are constructed from a variety of polymeric substances, including polyester, polycarbonate, cellulose nitrate, and cellulose diacetate. Gradocol (graded colloidion) membrane, an older type of membrane, was made of cellulose nitrate. Gradocol membranes typically have pores that are 3 to 10 m in size. The more recent ones contain cellulose diacetate. The pore sizes of these membranes range from 0.015 mm to 12 mm. Autoclaving is used to sterilise these filters. The two methods for creating membrane filters are forced solvent evaporation from cellulose esters to create labyrinthine pore membranes and radiation for capillary pore membranes.

The shortcomings of depth filters include the migration of filter material into the filtrate, the absorption or retention of a specific volume of liquid by the filters, the ambiguity of the pore sizes, and the potential for the passage of viruses and mycoplasma. Membrane filters have known porosity, no fluid retention, are reusable after autoclaving, and are chemically compatible. Membrane filters are fragile and have a low loading capacity. HEPA (High Efficiency Particle Air) filters can be used to filter the air. Biological safety cabinets are where they are typically used. At least 99.97% of particles larger than 0.3 m can be removed by HEPA filters. Rooms housing seriously neutropenia patients and operating rooms used for orthopaedic implant procedures are two examples of places where HEPA filters are used. The dioctylphthalate (DOP) particle test uses particles with a diameter of 0.3 m to measure the effectiveness of HEPA filters.

Ultrasound and sonic vibrations: On exposure for an hour, sound waves with a frequency >20,000 cycles per second kill some viruses and some bacteria. The killing effect of microwaves is primarily caused by the heat they produce; microwaves are not particularly antimicrobial in nature. Cells are damaged by high frequency sound waves. They are used to lessen microbial load as well as clean and disinfect equipment. Since many viruses and phages are not impacted by these waves, this method is unreliable.

Chemical methods of disinfection

Chemicals known as disinfectants are used to remove pathogenic bacteria from inanimate objects. Some chemicals have very narrow and very wide activity spectra, respectively. Chemisterilants are substances that have the ability to sterilise. Antiseptics are substances that can be applied to the skin and mucous membranes without causing harm. The following qualities an ideal antiseptic or disinfectant should have:

The most common sterilisation method is heating sterilisation because it completely destroys all microorganisms. The length of heating increases as temperature rises, and both factors have an

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effect on the degree of sterilisation. Two additional categories can be made for the heat sterilisation technique. In this case, heat is produced by boiling in addition to other methods like pasteurisation and the use of steam. Surgical scissors, personalised trays, and needles are among the metal items that are boiled. The ingredients are cooked in order to kill any microorganisms. As opposed to this, pasteurisation involves heating milk three or four times to 60 or 72 degrees. The autoclave steam heating apparatus exposes the items that need to be sterilised to steam. The procedure lasts for an hour at temperatures of up to 115 degrees. It is the most popular method for sterilising pharmaceuticals because it can eliminate bacterial spores, which are inactive bacterial forms.

Materials can be treated using burning, cremation, hot air ovens, or radiation sterilisation. Flaming is the practise of exposing metallic objects, such as scalpels or needles, to a flame for an extended period of time. The flame will instantly kill all bacteria. Incineration is used, particularly for inoculating the loops used in microbe cultures. When the metallic end of the loop is burned intensely on a flame, all microorganisms are destroyed. The radiation technique exposes packets of materials to radiation. The two types of radiation are ionising radiation and non-ionizing radiation sterilisation. The former is risk-free for the person administering the treatment, whereas the latter requires the operator to wear protective gear. With the hot air method, dry materials like glass and powder work best. They are heated on racks in a hot air oven until cleaned.

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CHAPTER 3

Culture Medium

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A growth medium, also known as a culture medium, is a solid, liquid, or semi-solid that is intended to foster the development of a population of microorganisms, cells, or miniature plants, such as the moss *Physcomitrella patens*, via the process of cell proliferation. Different cell types are grown in various kinds of medium. The two primary categories of growth media are those used in cell culture, which use certain cell types obtained from plants or animals, and those used in microbiological culture, which are used to grow microorganisms like bacteria or fungus. Nutrient broths and agar plates are the most typical growth medium for microorganisms; nonetheless, the development of microorganisms and cell cultures might sometimes need the use of specialised media. Due to their intricate dietary needs, certain organisms—referred to as fastidious organisms—need specific settings. For instance, viruses are necessary intracellular parasites that need a growing substrate with live cells.

Media should always be kept in a cold, moist environment to minimise evaporation, ideally in tubes or bottles with screw-top lids. However, it is not advised to keep sterile media for an extended period of time unless stability has been shown. Media tubes should be warmed just before use if they have been stored for any period of time. To release dissolved gases, liquid media should be heated for a short time in a boiling water bath or in flowing steam, and then immediately cooled in cold water without agitation soon before inoculation. All bacteria prefer a damp surface, thus agar tubes should be melted and then allowed to harden. These precautions, which apply to both liquid and solid media, are crucial for the development of highly parasitic organisms, such those seen in blood culture work.

Variety of Media

The two primary types of culture media are complicated (undefined) and synthetic (defined). All of the ingredients in a defined medium are known to the researcher, such as in a synthetic medium with glucose serving as the only carbon source, inorganic salts serving as sources of sodium, phosphate, and numerous other essential minerals like Fe++ or Mg++, and an ammonium salt serving as a source of nitrogen. While some bacteria can thrive on the medium mentioned above, others need growth factors that they are unable to produce on their own (i.e., they are fastidious). Extracts of plant or animal tissues, such as yeast autolysate or beef extract, are included in complex media. In the form of lipids, hydrolyzed proteins (a source of nitrogen as amino acids), carbon sources, vitamins, and other cofactors, these extracts provide a wide range of nutrients. Any media containing these extracts is referred to be undefined since it is uncertain what exactly makes them up. Other sources of these vital development hormones include whole blood, serum, infusions of brain or heart tissue, etc.

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Nutrient broths or lysogeny broth medium are the two most popular growth mediums for microorganisms. Agar is often added to liquid media before being placed onto Petri plates using a sterile media dispenser to harden it. These agar plates provide a stable substrate for the cultivation of bacteria. They are still solid because relatively few bacteria can break down agar (the exception being some species in the genera: Cytophaga, Flavobacterium, Bacillus, Pseudomonas, and Alcaligenes). Colloidal suspensions of bacteria are often formed in liquid cultures. Cells generated from complete organisms that are produced in culture often cannot develop without the inclusion of, for example, hormones or growth factors that typically occur in vivo, which is the difference between growth media used for cell culture and those used for microbiological culture. When dealing with animal cells, this problem is often solved by adding blood serum or a synthetic serum substitute to the media. Since microbes are often unicellular creatures, there are no such restrictions in the case of microorganisms. Animal cells in culture are typically grown on a flat surface on which they adhere, and the media is supplied in a liquid state, which covers the cells. This is another significant distinction. On the other hand, liquid or solid medium may be used to grow bacteria like Escherichia coli.

The difference between defined and undefined media is a crucial one when comparing various growth media types. All components in a specified media will be present in predictable amounts. They consist of giving microorganisms the trace elements, vitamins, and particularly designated nitrogen and carbon sources that they need. As inorganic nitrogen sources, ammonium salts or nitrates are often utilised, and as carbon sources, glucose or glycerol. Complex substances, like yeast extract or casein hydrolysate, which are made up of a variety of chemical species in an undetermined ratio, are present in an indeterminate medium. Some bacteria have never been cultivated on specified media, hence undefined media are sometimes selected based on price and occasionally out of need. It is necessary to use a culture medium, which may be either a gel or a liquid, to grow bacteria or other microorganisms. They are sometimes referred to as growth media. Different cell types are grown on various medium types. Nutrient broths and agar plates are the two categories of growth media that microorganisms utilise the most commonly. Some bacteria or germs need specialised media to develop.

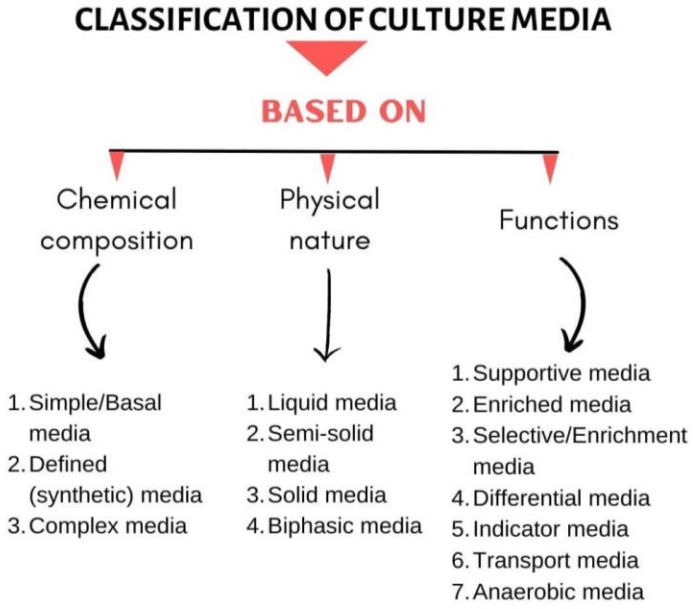

Figure 3.1: Classification of culture media

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It is required to recreate the environment or habitat that the microorganisms could encounter in the wild in order to develop them in the laboratory. Making culture media appropriate for the microorganisms helps with this. Scientists thus developed a number of culture media dependent on the microflora that would be propagated. In addition to trace components, the basic medium also contains a source of nitrogen, energy, and carbon growth stimulants. The ingredients that are often employed include peptone, water, casein hydrolysate, agar, meat extract, yeast extract, and malt extract. The medium's pH has to be properly adjusted as well. Its usefulness in the diagnosis of infectious disorders is one of the most significant justifications for in vitro bacterial culture. A bacterium's involvement in the development of a disease may be inferred from its isolation from areas of the body that are often thought to be sterile. Bacteria must first be cultured in order to be studied for morphology and identification. Antigens from bacteria must be grown in order to create vaccinations or serological tests. Bacteria must also be cultivated in vitro for a number of genetic experiments and cell modifications. A good method for calculating the amount of bacteria is to cultivate them (viable count). Another simple method for sorting bacteria in mixtures is to cultivate on solid medium.

History: Louis Pasteur employed straightforward broths produced from meat or urine extracts. In order to cultivate germs, Robert Koch utilised bits of potato after realising the value of solid medium. Agar was employed to cement cultural media upon the idea of Walther Hesse's wife Fannie Eilshemius, who worked as Robert Koch's secretary. Agar was first used after efforts to employ gelatin as a hardening agent. Gelatin has some inherent issues since some bacteria could digest it and it persisted as a liquid at standard incubation conditions. Chemoorganoheterotrophs are bacteria that infect humans (commensals or diseases). Similar environmental and dietary conditions to those found in the bacterium's native habitat must be provided for cultivating the bacteria. As a result, an artificial culture medium has to include every nutrient that a bacteria receives in its natural environment. Water, a supply of carbon and energy, a source of nitrogen, trace elements, and certain growth agents are often found in culture mediums. In addition to this, the pH of the medium must be adjusted appropriately. Agar, peptone, casein hydrolysate, meat extract, yeast extract, and malt extract are some of the components of culture medium. Water is another.

Bacterial culture medium may be categorised in at least three different ways: based on consistency, based on nutritional content, and based on functional use. Based on uniformity in classification: Biphasic culture medium come in liquid, semi-solid, or solid forms.

A) Liquid media are available for use in flasks, test tubes, and bottles. "Broths" is another term for liquid media (e.g nutrient broth). Bacteria grow equally and produce widespread turbidity in a liquid media. A thin film known as a "surface pellicle" of some aerobic bacteria and those with fimbriae (Vibrio & Bacillus) is known to form on the surface of an undisturbed broth. On ghee-containing broth, Bacillus anthracis has been seen to cause stalactite development. the original turbidity sometimes. When cultivated in liquid medium, long chains of streptococci have a tendency to tangle and deposit as granular deposits at the bottom. When a lot of germs need to be cultivated, liquid media are often employed. When it is believed that there are not many bacteria in the inoculum, they are ideal for growing bacteria. Any inhibitors of bacterial growth are also diluted by inoculating in a liquid media. In blood cultures, this is the sensible course of action. To determine the viable count, culture organisms in liquid media (dilution methods). Liquid media do not allow for the observation of bacterial characteristics or the detection of the coexistence of different bacterial species.

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STABLE MEDIA: By adding certain solidifying chemicals, any liquid media may be made solid. The most often used solidifying agent also known as agar). It is an unbranched polysaccharide that may be found in the cell membranes of some species of red algae, including those in the genus Gelidium. Agarose and agarapectin, which together make about 70% of agar, are two long-chain polysaccharides. It gels at 42 °C and melts at 95 °C, has minimal nutritional value, is seldom digested by most bacteria, and is often devoid of growth-promoting or growth-retarding chemicals. It melts at 95 °C (sol) and solidifies at 42 °C (gel). It could, however, be a source of calcium and organic ions. To create a solid agar medium, it is most often employed at concentrations of 1-3%. The New Zealand agar can gel faster than the Japanese agar. Agar may be purchased as powder or as fibres (shreds).

Agar that's still liquid but not quite solid A semi-solid medium is produced by lowering the agar content to 0.2-0.5%. These relatively soft medium (U-tube and Cragie's tube) may be used to show bacterial movement and distinguish between motile and non-motile strains. Some transportation media, including Stuart's and Amies' media, have a semi-solid consistency. Both the mannitol motility medium and Hugh & Leifson's test medium for oxidative fermentation are semi-solid.

PHASIC MEDIA: It's fairly uncommon for a culture system to have both liquid and solid media in the same container. Biphasic medium is what this is (Castaneda system for blood culture). When creating subcultures, the inoculum is put to the liquid medium, and the container is simply rotated to enable the liquid to flow over the solid media. This eliminates the need to often open the culture bottle for subculture. In addition to agar, culture medium may also be solidified using egg yolk and serum. Normal liquids like serum and egg yolk may be turned into solids through a process called coagulation. The process of inspissation solidifies and sanitises egg and serum-containing media, including Loeffler's serum slope, Lowenstein Jensen medium, and Dorset egg medium.

Using nutritional components to classify: Simple, complicated, and synthetic media may all be categorised (or defined). Some bacteria need additional nutrients, even if the majority of the nutritional elements are the same across medium. Bacteria that can thrive with little support are referred to as non-fastidious, whilst those that need additional nutrients are referred to as fastidious. The majority of non-fastidious bacteria can be supported by simple media like peptone water and nutrient agar. It is challenging to determine the precise composition of the constituents in complex medium like blood agar. Research-specific media with well-established component compositions are known as synthetic or defined media, such as Davis & Mingioli medium.

Classification based on application or functional use: Indicator/differential media, basal media, enriched media, selective/enrichment media, transit media, and holding media are a few of these. Simple media, or basal media, are what most non-fastidious bacteria need to survive. Nutrient broth, nutrient agar, and peptone water are regarded as the basic media. Media that has been "enriched" by the addition of additional nutrients such as blood, serum, egg yolk, etc. to a base medium. Growing nutrient-demanding (picky) bacteria requires enriched medium. Some examples of enriched media are blood agar, chocolate agar, Loeffler's serum slope, etc.

In order to recover pathogen from a bacterial mixture, selective and enrichment media are used to prevent undesirable commensal or contaminating microorganisms. While enrichment media are liquid in nature, selective media are agar-based. These two mediums provide the same function. By adding certain inhibitory chemicals that don't harm the pathogen, any agar medium may be made selective. Addition of antibiotics, colours, chemicals, pH adjustment, or a combination of these are just a few methods for making a medium selective. Liquid media known as enrichment

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media are used in clinical specimens to suppress commensals. Pathogens are extracted from faecal specimens using selenite F broth, tetrathionate broth, and alkaline peptone water.

Indicator media or differential media: Different bacteria may be distinguished from one another based on the colour of their colonies in certain mediums. To make the bacteria that use them appear as distinct coloured colonies, other strategies include the addition of dyes, metabolic substrates, etc. These media are also known as indication media or differential media. Examples include XLD agar, MacConkey's agar, CLED agar, and TCBS agar.

Transport media: To avoid the proliferation of commensals or contaminating organisms, clinical specimens must be transferred right away from the point of collection to the lab. Utilizing a transfer medium may help with this. Such media preserve the pathogen to commensal ratio, avoid drying (desiccation) of specimens, and restrict bacterial expansion. The consistency of some of these media (Stuart's & Amie's) is semi-solid. Charcoal is used to help counteract restricting elements. The faeces of cholera suspects are transported via the Cary Blair and Venkatraman Ramakrishnan mediums. To transport patient faeces suspected of having bacillary dysentery, Sach's buffered glycerol saline is employed.

Anaerobic medium: In order to thrive, anaerobic bacteria need specialised media that have low oxygen concentration, a low oxidation-reduction potential, and additional nutrients. Nutrients like hemin and vitamin C may need to be added to media for anaerobes. Any dissolved oxygen is removed by boiling the medium. Any of the following may decrease a medium: 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine, or red-hot iron filings. Robertson prepared meat often used to increase size. A 2.5 cm column of bullock heart meat and 15 cc of nutritional broth are both present in the medium-sized Clostridium spp. Prior to usage, the medium must be sterilised liquid paraffin before being cooked in a water bath to remove any dissolved oxygen. The thioglycollate medium contains methylene blue, also known as resazurin, which is an indication of the oxidation-reduction potential. Methylene blue is colourless when it is decreased.

Before autoclaving, the medium's pH has to be carefully adjusted. There are several different pH indicators in use, such as phenol red, neutral red, bromothymol blue, bromocresol purple, etc. Commercially available dehydrated media must be reconstituted in accordance with the instructions provided by the producer. By autoclaving, most cultural materials are made sterile. Some media, such as glucose, antibiotics, urea, serum, and blood, include heat-labile components that are not autoclavable. Following the autoclaving of the medium, these parts may be introduced individually after being filtered. It is not necessary to sterilise certain highly selective media, such as TCBS agar and Wilson and Blair's medium. Prior to usage, a sample from each batch must be examined for functionality and contamination. Once prepared, media may be kept in the fridge at 4-5o C for 1-2 weeks. It is possible to store certain liquid media at room temperature for weeks in screw-capped bottles, tubes, or cotton plugs.

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CHAPTER 4

Control of Microorganisms

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Controlling microorganisms is essential for slowing deterioration and spoilage, avoiding unfavorable microbial contamination, and reducing the spread of diseases and infections. Treatments that are chemical or physical may be used to control microorganisms. Radiation, filtration, desiccation, high or low temperatures, and osmotic pressure are a few examples of physical agents and methods for control. Control by chemical agents involves the use of antimicrobial chemotherapeutic chemicals, antibiotics, antiseptics, disinfectants, and other similar substances. The primary subject of this section will be the chemical control of microbial growth, with an emphasis on the antibiotics and chemotherapeutic antimicrobial substances used to treat bacterial infections. It is important to regulate the development and quantity of microorganisms in or on a variety of commonly used human objects in order to stop the spread of human illness. The term "fomites" refers to inanimate objects like toys, towels, and doorknobs that may house bacteria and spread illness. The degree of cleanliness necessary for a certain fomite and, therefore, the technique used to reach this level, are strongly influenced by two considerations. The use that the object will be put to as the first consideration. A significantly higher standard of cleanliness is needed for invasive applications than for non-invasive ones, for instance, as they must be inserted into the human body. The amount of resistance that possible bacteria have to antibiotic therapy is the second consideration. For instance, foods that are canned often pick up the neurotoxin that causes botulism, produced by the bacteria Clostridium botulinum. Extreme pressures and temperatures are required to destroy the endospores produced by C. botulinum since they can withstand severe environmental conditions. Other creatures may not need to be controlled with such drastic tactics, and could instead respond to a process like running a load of laundry through the washing machine.

Biosafety levels in laboratories

The hazards connected to certain diseases establish the necessary standards of cleanliness and control for researchers or laboratory staff dealing with pathogens. The "biological safety levels" are four categorization levels that have been created by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) (BSLs). Similar categorization systems are used by other international organisations, such as the World Health Organization (WHO) and the European Union (EU). The infectivity, ease of transmission, probable illness severity, and kind of job being done with the agent are all taken into account when determining the BSL, according to the CDC. The amount of biocontainment needed for each BSL varies in order to stop contamination and the transmission of infectious agents to lab workers and,

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eventually, the general public. For instance, BSL-1, the lowest BSL, corresponds to settings with the lowest risk of microbial infection and so calls for the fewest precautions.

Agents classified as BSL-1 are those that typically do not infect healthy adult humans. These include viruses like baculoviruses that are known to infect animals besides humans as well as non-pathogenic forms of bacteria like Escherichia coli and *Bacillus subtilis* (insect viruses). There are extremely few safety measures required while dealing with BSL-1 compounds since the risks are so low. Working with these agents at an open laboratory bench or table while using PPE such a lab coat, goggles, and gloves as necessary, laboratory personnel employ normal aseptic procedure. There isn't anything else that has to be changed, save a basin for handwashing and doors to divide the laboratory from the rest of the building.

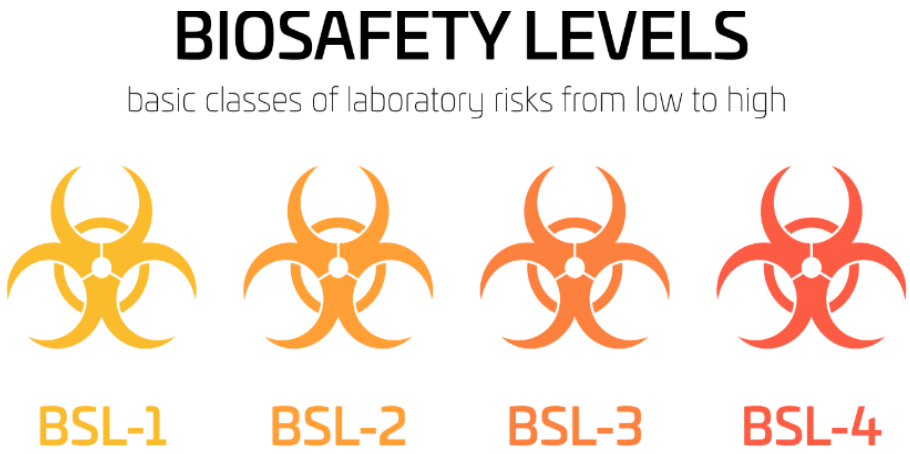

Figure 4.1: Categories of Biosafety levels

Agents with a BSL-2 classification include those that are "indigenous," or normally present in that region, and pose a moderate danger to laboratory personnel as well as the general public. These include viruses such as the hepatitis, mumps, and measles viruses as well as bacteria such as Salmonella spp., Staphylococcus aureus, and other related species. Restricted access, required personal protective equipment (PPE), which may include a face shield in some situations, and the use of biological safety cabinets for procedures that might disperse agents through the air (referred to as "aerosolization") are all prerequisites for BSL-2 laboratories. These precautions go above and beyond those of BSL-1 laboratories. Self-closing doors, an eyewash station, and an autoclave, a specialised tool for sterilising materials with pressured steam before use or disposal, are all included in the BSL-2 laboratory' equipment. Autoclaves are also possible in BSL-1 labs.

By inhalation, BSL-3 toxins have the capacity to spread deadly illnesses. These include diseases like Mycobacterium TB, Bacillus anthracis, West Nile virus, and human immunodeficiency virus, and they may either be local or "exotic," which means they came from a different country or region (HIV). Restricted access is needed for labs dealing with BSL-3 agents because of the devastating illnesses that these agents may cause. Employees in laboratories are subject to medical examinations and may be immunised against the germs they handle. Employees at BSL-3 labs are required to wear respirators when working with germs and infectious agents at all times in a biological safety cabinet in addition to the usual PPE previously specified. An eyewash station close to the exit, two sets of self-closing, locking doors at the entry, and a hands-free sink are required in BSL-3 labs. As a result of the directed airflow that is present in these labs, pure air is

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drawn from clean regions to possibly polluted ones. It's necessary to have a steady flow of clean air since this air cannot be recycled.

The deadliest agents are those with a BSL-4 rating. These microorganisms often cause diseases for which there are no cures or vaccines, are readily spread by inhalation, and are unusual in appearance. Viruses that cause hemorrhagic fevers include the Marburg and Ebola viruses as well as the smallpox virus. The United States and other countries only have a tiny number of labs that are properly set up to deal with these substances. Laboratories at BSL-4 facilities additionally require that staff change into clean clothes before entering the lab, take a shower after leaving, and disinfect all equipment before leaving. They must either conduct all work in a biological safety cabinet with a high-efficiency particulate air (HEPA)-filtered air supply and a double HEPA-filtered exhaust or wear a full-body protective suit with a designated air supply while doing laboratory work. If you're wearing a suit, make sure the air pressure inside is greater than the air pressure outside so that if there is a breach, polluted lab air won't be dragged inside. The laboratory must have its own air supply, exhaust system, decontamination system, and be situated either in a separate building or in a remote area of a building. In figure 13.3, the BSLs are enumerated.

Chemotherapeutic bacterial control is based on selective toxicity. In accordance with the theory of "selective toxicity," the chemical used should either inhibit or eradicate the targeted illness without negatively affecting the host. A broad-spectrum agent is one that often works well against a variety of Gram-positive and Gram-negative bacteria, whereas a limited spectrum agent frequently only works well against Gram-positives, Gram-negatives, or a small number of bacteria. As previously mentioned, these compounds may function in a cidal or static way. While a static agent prevents the organism's growth long enough for the body's defences to get rid of it, a cidal agent destroys the organism. The two main chemotherapeutic approaches for treating bacteria are antibiotics and synthetic drugs. The metabolic byproducts of one kind of microbe are antibiotics, which may either stop or kill the growth of other bacteria. Chemotherapeutic synthetic pharmaceuticals, which are used to make antimicrobial drugs, are created in laboratories utilising chemical procedures. More often, synthetic materials are used in the production of antibiotics; some are even entirely synthetic. Byproducts from the metabolism of one microbe called antibiotics may stop or kill other germs. What causes bacteria to produce antibiotics in light of this? The following techniques are becoming more popular for producing microbial antibiotics:

If enough antibiotics are produced, they may be used as a weapon to kill or inhibit other surrounding microbes in order to reduce competition for food. A variety of bacteria may live in a single biofilm where one organism's metabolic byproducts may be utilised as a substrate for another thanks to the interspecies quorum sensing abilities of sublethal levels of antibiotic synthesis. Each animal is safeguarded inside the same biofilm. Antibiotics produced in low-lethal levels may work as quorum-sensing substances that enable certain bacteria to tell other microbes to become motile and swim away, reducing competition for food. Antibiotics may break down bacterial cell walls or DNA, and these remnants may function as cues for other microbes to form a protective biofilm. A species may experience quorum sensing as a result of the synthesis of sub-lethal antibiotics. Bacteria may produce quorum sensing molecules as a result of low levels of antimicrobial exposure, which in turn may encourage the development of a protective biofilm by the population. A biofilm then protects the population from greater antibiotic concentrations. Antimicrobial byproducts are substances produced by the metabolism of one microbe that stop or get rid of other germs. What causes bacteria to produce antibiotics in light of this.

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How Chemical Control Agents Affect Bacteria

Chemotherapeutic control of microorganisms is based on selective toxicity. Selective toxicity is the ability of a chemical to kill or suppress the targeted pathogen with little to no noticeable harm to the host. A broad-spectrum medication typically combats both Gram-positive and Gram-negative bacteria, while a narrow spectrum medication frequently only combats Gram-positives, Gram-negatives, or a small number of bacteria. These substances can exhibit either a cidal or a static behaviour. The normal production of peptidoglycan by bacteria is inhibited by a number of antibiotics, such as cephalosporins, penicillins, and bacitracin, which results in osmotic lysis. In order to produce peptidoglycans, transporters or an enzyme must be inactive, which is what antibiotics do. Several antibacterial chemotherapy medications prevent the normal formation of the acid-fast cell wall (INH, ethambutol). Only a small number of antibiotics (colistin, polymyxin, and daptomycin) cause the bacterial membranes to rupture, allowing chemicals and enzymes necessary for normal bacterial metabolism to leak out.

A few antimicrobial chemotherapeutic drugs that stop bacteria from reproducing their genetic material normally include sulfonamides, fluoroquinolones, and trimethoprim. Numerous antibiotics, such as tetracyclines, macrolides, oxazolidinones, and streptogramins, alter bacterial ribosomes, impairing the translation of messenger RNA (mRNA) into proteins and resulting in improper protein synthesis. Sanitizers, disinfectants, and sterilisers all function by either causing damage to the bacteria's semipermeable cell membrane's triglycerides and/or protein, which allows cellular contents to leak out, or by denaturing the enzymes and other proteins that are present in the microorganisms. Several factors, including the chemical concentration of the agent, the application temperature, the kinds and numbers of bacteria present, and the composition of the substance containing the microorganisms, can affect these's capacity to kill microorganisms. Endospore-producing bacteria like Mycobacterium TB, Bacillus species, and acid-fast bacteria are more challenging to eradicate.

Potential for Bacteria to Develop Chemical Control Agent Resistance

The majority of bacteria become antibiotic-resistant through one or more processes that are controlled by genes on their chromosomes and/or plasmids. Bacteria may use its genes to produce an enzyme that renders the antibiotic inactive. Bacterial genes may alter the ribosomal subunit, enzyme, or another target site receptor for the antibiotic to reduce or eliminate binding. It's possible that different membrane components are encoded by various bacterial genes. Alcohol, phenol and phenolic compounds, halogen compounds, heavy metals and their compounds, aldehydes, gaseous agents, detergents, and antibiotics are just a few examples. Alcohol is an antimicrobial substance. Alcohol's antimicrobial effect increases as its molecular weight rises. The alcohol that is most frequently used to control microorganisms is ethanol. Ethanol between 50 and 90% concentrations is effective against vegetative cells. 70% ethanol is used for practical reasons. Alcohol kills organisms by denaturing the proteins in their cells. The lipid bilayer of the cell membrane and cell wall are both damaged by alcohol, a lipid solvent. Additionally, it dehydrates the body and results in cell water loss. Alcohol is frequently used as a skin sanitizer, disinfectant for surgical instruments, and thermometer. Viruses can be killed by concentrations greater than 60%.

Alcohol solubilizes the lipid bilayer of the cell wall and membrane when used as a disinfectant, which results in pores. Through the pore, the remaining alcohol diffuses into the cytoplasm, where it denatures the cellular proteins and kills the bacteria. Absolute alcohol (alcohol at 100%) only results in bacteriostasis, so 70% alcohol is more effective than 100% alcohol. As alcohol

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concentration rises, its ability to cause denaturing, lipid solubilization, and dehydration also rises and become antagonistic to one another. Absolute alcohol causes extreme cell shrinkage and extreme dehydration, which prevents the cell from absorbing more alcohol. As a result, it only causes bacteriostasis. Although it almost achieves equilibrium, 70% alcohol is very effective at dehydrating and denaturing substances while also having bactericidal effects.

The phenolic compounds and phenol

The antimicrobial spectrum of phenol is very broad. While bacterial spore are resistant, vegetative cells are more and more quickly killed by concentrated aqueous phenol solution. The typical disinfectant used is a 2-5% aqueous solution of phenol. Because it is toxic and is absorbed through the skin and mucous membranes, phenol has a limited range of applications.

Several phenolic compound derivatives and their uses include:

i) Cresol (methyl phenol) is a cleaning agent used in lysol solutions to disinfect glassware and mop hospital rooms' floors.

ii) Chloroxylenol (dimethyl phenol), a key component of dettol and a popular antiseptic,

iii) Chlorohedidane (Hibitane): This substance, which is a part of Savlon, is used as an antiseptic in burns, wounds, and preoperative skin antisepsis.

Hexachlorophene, compound IV, is insoluble in water. It's utilised in soap. The killing effects of phenol and phenolic compounds on microorganisms include cell disruption, protein precipitation, enzyme inactivation, and leakage of cellular components.

Compounds with halogens

Iodine is a substance that can be found in a variety of forms, including tinctures, aqueous solutions, and iodophor. Both tincture and aqueous iodine have undesirable side effects like staining and irritation. As a result, Iodophore is now used as a replacement because it has fewer side effects. Iodine works well against all types of bacteria. It also has sporicidal properties. Iodine has some virucidal and potent fungicidal properties. Iodophore are frequently used as antiseptics for wounds, mucous membranes, and skin. The disinfection of water, air, and food utensils are just a few additional uses for iodine preparation. Iodine has strong oxidising properties and permanently oxidises the components of cells. Additionally, iodine causes the inactivation of the tyrosine residue found in enzymes and proteins.

Chlorine: As a disinfectant Chlorine is used in the forms of hypochlorite and chloramine. Because it is toxic and corrosive, free gaseous chlorine is challenging to handle. Both sodium hypochlorite and calcium hypochlorite are frequently employed. Household bleach is an aqueous solution of sodium hypochlorite (5.25%). Because chloramine is more stable than hypochlorite, it is more effective as a germicide. One of the most popular water disinfectants is chlorine. To disinfect kitchenware, use calcium hypochlorite. For personal hygiene purposes, such as bath water, 1% bleach is used. For swimming pool and household uses, bleach is used in concentrations of 5–12%.

Action mode: Free chlorine is released and turns into hypochlorous acid when hypochlorite or chloramine is added to water (HClO). Hypochlorous acid breaks down to release nascent oxygen, a potent oxidizer that destroys microorganisms by oxidising their cellular constituents. By directly

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chlorinating the proteins and enzymes, chlorine and chlorine-containing compounds also inactivate them.

Substances made up of heavy metals

Most heavy metals have an antimicrobial effect. Mercucry (Hg), Silver (Ag), and Cupper are the most popular and efficient materials (Cu). Cellular proteins and enzymes are directly combined with by heavy metals and their compounds, which renders them inactive. The cellular proteins coagulate and precipitate in high concentrations of heavy metal salts, killing the microorganisms. Among the frequently used metal compounds are: I HgCl and HgCl2, which are used as antiseptics in ointments. $AgNO_3$ is both bacteriostatic and bactericidal. To protect children from Ophthalmia neonatarum, it is included in eye drops.

Cupper Suphate: This substance is frequently used in swimming pools to combat mould and algae.

Aldehydes: Gluteraldehyde and formaldehyde are two of the most widely used aldehydes. Both are extremely sporicidal and microbicidal.

i. Formaldehyde: Only at higher temperatures and concentrations is formaldehyde stable. Para-formaldehyde was created when it polymerized at room temperature. Formaldehyde is used in two different forms, including formalin (a 40% solution of formaldehyde) and gaseous formaldehyde, to disinfect and sterilise enclosed spaces like operating rooms. The woollen blanket, wools, and footwear of a person with a fungal infection are also cleaned with formaldehyde vapour. The preservation of biological specimens involves the use of formalin.

ii. Gluteraldehyde is utilised in a 2% solution. It has similar antibacterial, antifungal, antisporal, and antiviral properties to formaldehyde. Both urological and respiratory therapy equipment is sterilised with gluterladehyde.

Gaseous agents

Common gaseous sterilizing agents include ethylene oxide, -propiolactone, and formaldehyde.

i. Above 10.8°C, ethylene oxide is a gas. Even endospores are killed by ethylene oxide's potent antimicrobial properties. Heat-sensitive materials like spices, oils, plastics, etc. are sterilised using this method. In the production of Freon, ethylene oxide is combined with CO2 (CClFe).

ii. Propiolactone is a gas that exists above 15.5 °C. Propiolactone has a lower penetration power than ethylene oxide, but it kills microorganisms more effectively.

It is not frequently used because of its cancer-causing properties.

Detergents

Detergents have antibacterial characteristics but are largely used for cleaning. Cationic, anionic, and non-ionic detergents are the three different kinds of detergents. Compared to the other two, cationic detergents are a more effective germicidal agent. A cationic cleaner with a germicidal effect is a quaternary ammonium compound. With Gram positive bacteria, it works better. Cleansers, disinfectants, and antiseptics are all uses for detergents. Additionally, they are used to clean hospital floors. By denaturing proteins and enzymes and obstructing glycolysis, detergents eliminate microbes from the environment. The cell membrane and wall are also damaged by

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detergents. Antibiotics are secondary metabolites that some microbes make that prevent the development of other germs. Different antibiotic subgroups work in various ways.

Penicillin and cephalosporin, for example, prevent the production of peptidoglycans in cell walls.

e.g., polymyxin, polyenes; inhibit cell membrane biosynthesis

e.g., chloramphenicol, tetracycline, inhibits protein synthesis

Quinolone and rifampin both react with nucleic acids

e.g., trimethoprim, sulfonamide, prevents the production of folic acid

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CHAPTER 5

Microbiological Cultures

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Koch, Pasteur, and his collaborators created media formulations in the late 19th and early 20th centuries utilising ingredients including cow brains, potatoes, grass, and a range of other enticing microbe feeds. Nowadays, it is possible to purchase bacteriological medium compositions in powder form; all that is needed for preparation is to weigh out the precise amount, add the required amount of water, and mix. The medium is sterilised in an autoclave, which produces steam under pressure and achieves temperatures above boiling, after the production of the fundamental formula. When sterilised medium has cooled, it is prepared for usage. Microbiological cultures are microbial organisms that have been replicated and grown using a predefined culture medium in a laboratory setting. For a microbiological culture to flourish, an environment with controlled pH, temperature, ambient gases, and pressure is required.

Proteins, salts, carbs, vitamins, and growth factors are additional necessities for the bacteria. Micro-organisms are grown for a variety of purposes, including diagnosing illness and determining an organism's species and presence in a collection. Pure cultures must be gathered in order to correctly identify the bacterium. Axenic cultures, also known as pure cultures, are those that thrive when there are no other species present. To produce pure culture, a number of methods are utilised. Let's review them fast.

Growing Microorganisms

A collection of laboratory-cultivated microorganisms is referred to as a culture. A pure culture only contains one kind of bacterium, but a mixed culture has two or more different kinds. When bacteria are grown on the same medium over an extended period of time, the nutrients are depleted and toxic byproducts are released, ultimately leading to the death of the whole colony. As a result, in order to sustain a growing bacterial population, bacterial cultures must be routinely transferred or subcultured to new media.

Microbiologists utilise subculturing techniques to produce and maintain bacterial cultures, examine cultures for purity or appearance, or count the number of live organisms. A method known as subculturing is used in clinical labs to establish pure cultures of infectious agents and for research projects aimed at identifying the organism. Since bacteria may exist almost everywhere, aseptic conditions must be maintained throughout subculturing techniques to avoid bacterial or fungal contamination of a priceless culture.

Fundamentally, aseptic methods in microbiology just need good judgement and adequate laboratory skills. First of all, keep in mind that every surface you come into touch with might be

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carrying germs. After that, think about what steps you may take to reduce your exposure to unwanted invisible intrusions. You should also think about ways to prevent your bacterial cultures from being contaminated by external germs (which includes you). To maintain an aseptic working environment, anything you handle should be initially microbe-free. Thus, before we begin, all of the glassware, pipettes, and culture tubes have been pre-sterilized. Inoculating needles and loops made of metal wire may be used to transfer bacteria from one medium to another and from the surface of an agar plate to a broth. Metal tools may be sterilised by being burned in a Bunsen burner flame. Glass tools, metal spreaders, or forceps that cannot be sterilised by direct heat are immersed in alcohol and quickly passed through a flame to speed the evaporation process. The lab will begin with a demonstration of typical aseptic techniques for growing bacteria.

Techniques for Bacterial Culture

Bacterial cultures may be grown using a liquid broth medium, such as Luria broth. For use in subsequent procedures, a significant number of microorganisms are cultivated in the broth in a flask. To maintain consistent growth, these cultures may either be agitated or put in a static incubator with an oxygen gradient.

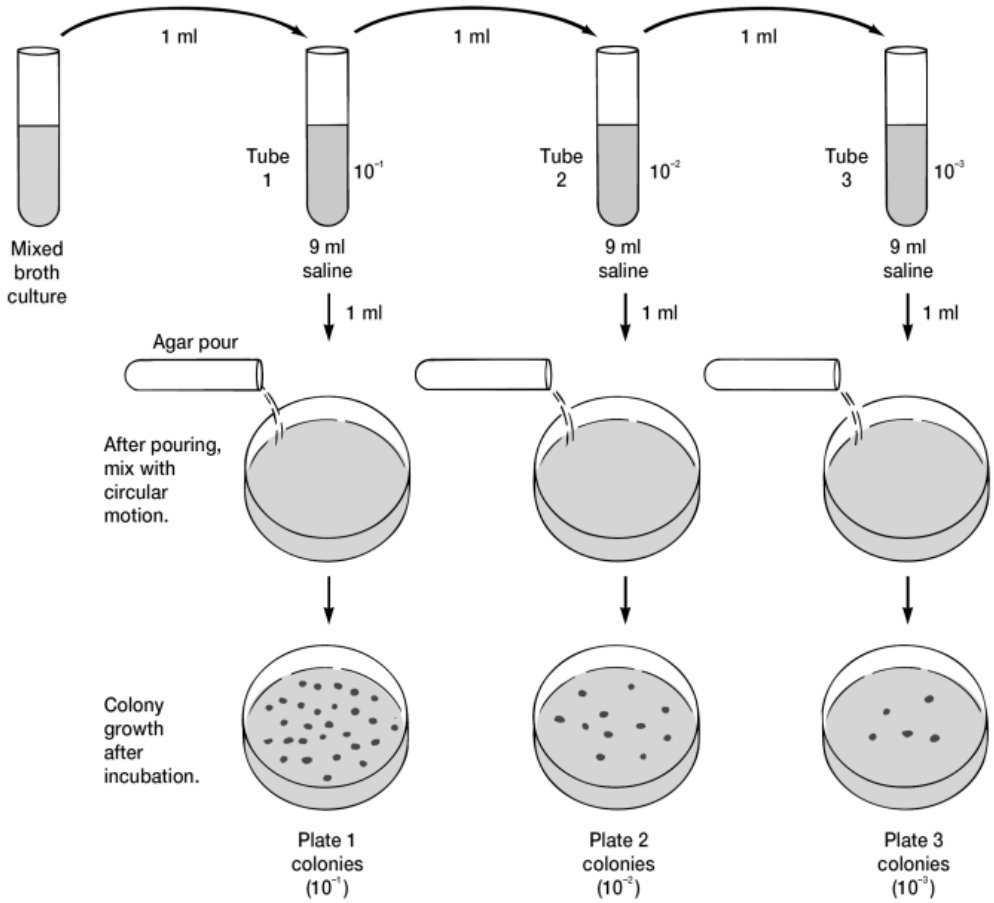

Figure 5.1: Microbial colonies identification through serial dilution method

Agar plates

In petri dishes with a thin layer of agar medium inside of them, researchers may establish bacterial cultures. The correct bacterial strain is put to the agar plates, which are then maintained overnight

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at the ideal temperature for the growth of the bacteria in an incubator. After achieving their maximal growth, the agar plates may be wrapped and stored for further uses inverted in the refrigerator.

Before being placed onto a plate and allowed to set, agar may have a variety of additives added to it. Some bacteria can only grow in the presence of specific substances. Making genetically altered bacteria strains with an antibiotic resistance gene also entails doing this. When the selected antibiotic is applied to the agar, only bacterial cells that contain the resistance-granting gene insert will be able to grow. The researcher may then choose just the populations that successfully experienced a transition. The most common solid foundation for growing bacteria is agar plates. Agar keeps the microbial growth medium in a semi-solid, gel-like form. Microbial growth media also includes nutrients and an energy source to power the microorganisms as they proliferate. A single microbe will multiply and thrive on solid medium to form a "colony," or group of identical offspring. Microbiologists may tell whether a culture is pure or distinguish the kinds of bacteria in a mixed sample by looking at the form, colour, and texture of the colonies that various types of microorganisms create. Premade plates are offered by many biological supply firms, however creating your own is far less costly. Making your own plates will become extremely simple for you with a little experience, and you'll have the extra flexibility of being able to modify recipes to meet your requirements.

1. Select a dish

Either use a recipe you create yourself or one from the Media Recipes page. Determine the number of plates you'll need. Our recipes may be adjusted up or down as required. One L (1000 mL) of media from our recipes can fill around forty 100 mm plates. Per 100 mm plate, allow 25 mL to be used.

2. Compile resources

Media ingredients for recipes (see the Media Recipes page). Sterile Petri dishes made of polystyrene. The most popular size is 100 x 15 mm, although there are also 60 and 35 mm sizes. Moreover, use a glass jar that can store at least twice as much media. When sterilizing your media in an autoclave, pressure cooker, microwave, or hot plate, cover the media container with aluminum foil or plastic wrap (see the Sterilizing Liquids page). hothands, potholders, or heat-resistant gloves for handling hot containers. Cleaning your workspace using 70% ethyl or isopropyl (rubbing) alcohol, 10% bleach, or disinfectant wipes. Equipment for handling solid substances include a graduated cylinder for measuring water and a balance for weighing ingredients (such as weigh boats and scoopulas, or paper plates and spoons)

3. Set up the media

Use a glass container that can hold at least twice as much media (preferably an Erlenmeyer flask). Put the ingredients together in accordance with the recipe of your choosing. If you use a microwave, cover with aluminum foil or plastic wrap.

4. Cleanse

Utilize one of the sterilisation techniques listed on the page for sterilising liquids. Because normal contaminating bacteria can't thrive on high-salt medium, boiling may be used to sterilise media with a salt content of at least 10%. Verify that the agar has fully dissolved. The agar could take

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longer to dissolve in conditions that contain 15% or more salt. You could see little, transparent lens-like things floating in the media or a foggy appearance. Boil the medium continuously until it is fully clear; this might take more than 15 minutes. Your medium will become flimsy or floppy if the agar isn't entirely dissolved.

5. Transfer to plates.

Having trouble finding a lengthy period of undisturbed time? Prepare your solid media in bottles, disinfect it, and then allow it to totally cool there. You may reheat the media at a later time by heating it in a hot water bath or a microwave until it melts. Pour it onto plates after allowing it to cool to a touchable temperature. Establish a convenient workspace. (For further information, check the Sterile Technique page.) The kind of media you will pour into the plates should be marked on the plates. Stir the heated media briskly to combine. The medium should be cooled for 20 to 30 minutes, or until it is just cold enough to handle. For a few seconds, you should be able to hold your hand against the container rather comfortably. The medium will begin to harden in the container if it is too cold. Excessive condensation will remain on the lids if it is too hot. Just before pouring, stir the media one more to combine; be cautious not to integrate bubbles. 25 mL or so should be poured onto the dish to completely cover the bottom. Place the plates in a plastic bag or the plastic sleeve they came in after a few hours or overnight. Label the bag with the date and the media type, then put it upside-down in the fridge. The plates may be kept in the fridge for 4-6 weeks.

Dipsticks made with agar

These little agar plates are used for diagnostics and are arranged like dipsticks. One of its key advantages is the absence of reliance on a laboratory environment or a skilled expert.

Cultures Stab

By putting the agar medium into a test tube and allowing it to set, agar plates are a kind of culture. A pipette or a needle is used to inject the medium after making a hole in the agar. This method is often used to move cultures or preserve them briefly.

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CHAPTER 6

Isolation of Bacteria

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Bacteriological research begins with bacterial isolation, purification, and identification. To get pure bacterial cultures, isolation is carried out. Typically, bacteria are isolated from fish kidney and spleen, as well as shrimp hepatopancreas, lymphoid organ, and muscles. These tissues serve as monitoring organs and typically hold the pathogenic germs throughout an illness. The initial step in identifying bacteria is to create a pure bacterial culture. The study of a specific bacterial strain's appearance, physiology, metabolic traits, and sensitivity to antimicrobial treatments requires pure culture. The best way to get pure cultures is by utilising solid medium, either via the streak plate or pour plate approach. If done correctly, streak plate is the most useful technique.

A loopful of the inoculum is put close to the edge of the plate with agar medium and distributed or streaked across the top part of the plate with parallel overlapping strokes in the streak plate technique. In order to see separate colonies in the final streaked region, the inoculum was streaked across various areas of the plate. In fish diagnostics, a bacterial pathogen's identification is crucial. Only once the bacteria or causal agent has been identified can treatment be started. Bacterial species may be coded or labelled using differences in their morphological, physiological, and biochemical properties. Therefore, a variety of physical, physiological, and biochemical tests are used to identify people. These tests' findings are contrasted with recognised taxa or identification systems. Bacterial cultures need to be stored for future research. Bacterial cultures are preserved when stored in the right media. The easiest technique is to sub-cultivate the organism or shift it to a new solid medium with little to no nutrients to avoid bacterial proliferation. The bacteria are either wrapped with paraffin oil or kept at room temperature in the dark after being given time to proliferate before being stored there. Another straightforward procedure involves deep-freezing the bacterial culture that has been kept in a glycerol-rich broth medium.

To stop bacterial cells from desiccating, glycerol is introduced. Additionally, bacterial cultures may be stored using lyophilization or freeze-drying. Using this technique, water is sublimated under vacuum out of the frozen bacterial solution. Before being stored, bacterial cultures should be appropriately labelled or labelled. When labelling a tube or vial to hold bacterial cultures, it's crucial to use permanent ink. The reference number and other important details like the source of the sample (host animal, location), date of isolation, special qualities, identity, name of the person who isolated the organism, and the date that the stock culture was prepared should all be included on the label or code. Microbes come in 10,000 distinct varieties, according to research. For every recognised species, there are thought to be 10,000–100,000 more unknown species. Identification of microorganisms is important in many different fields of microbiology. For instance, in food microbiology, it is essential to be able to precisely identify the microorganisms that cause food to

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rot. Identification of micro-organisms aids in characterising the variety in microbial communities. The primary goals of medical microbiology, a branch of microbiology that studies pathogenic microorganisms, are the isolation, identification, and study of disease-causing bacteria.

Microorganism In order to determine if an organism is beneficial or harmful and whether it causes disease, identification aids in designating the organism down to the genus or species level. The initial step in identifying the microorganism, such as bacteria, that may be responsible for a disease is the isolation of a specific bacterial species. Identification is necessary so that the disease or illness this bacterium causes may be treated with the right drugs. Microorganisms may be found in the soil, which is a natural environment. They cohabit with a variety of other living forms. There are numerous dangerous microbes. They may cause a variety of diseases with a range of symptoms, depending on how they interact with the patient. A suspicious bacteria must be isolated and given space to grow in pure culture in order to detect and treat the infectious pathogen.

The primary culture from a natural source is often a mixed culture made up of many species of microorganisms. But in a lab, several species may be maintained separate. One kind of bacterium can only be found in a pure culture. The process of separating one species of microbe from a mixture of other species to establish a pure culture is known as "isolation of the organisms." Utilizing specialised techniques, microorganism pure cultures are produced. Direct transfer or isolation may sometimes retain a pure culture. Only environments where pure culture naturally develops are capable of enabling this. The sorts of specimens utilised for cultivation will change based on the microorganisms and their surroundings. The following methods of isolation are used to isolate bacteria from mixed cultures. Streaking, plating, dilution, enriched method, single cell technique, and others

Streaking

Streaking is a method used in microbiology to separate a pure strain from a single species of microbe, often a bacterium. In order to identify, research, or test the organism, samples may be obtained from the resultant colonies and a microbiological culture can be produced on a new plate. Since Robert Koch and other microbiologists sought for bacterial microbiological cultures in order to investigate them, the contemporary streak plate approach has advanced. Initially devised by Loeffler and Gaffky at Koch's lab, the dilution or isolation by streaking technique involves methodically streaking bacteria across the surface of the agar in a Petri dish to create isolated colonies that would later expand into large numbers of cells, or isolated colonies. The culture is regarded as a microbiological culture if the agar surface supports the growth of microorganisms that are all genetically identical.

Streaking is a quick and, ideally, straightforward isolation dilution technique. The method involves reducing the concentration of bacteria from a relatively high concentration to a lower one. Bacterial decline should indicate that colonies are sufficiently spaced apart to influence the separation of the various microbe kinds. Streaking is done using a clean implement, usually an inoculation loop or a cotton swab. Microbiological cultures are kept up-to-date using aseptic procedures, which also help to keep the growing media clean. The process of streaking a plate may be done in a variety of ways. The approach used depends on personal choice as well as the quantity of microorganisms present in the sample. Beginners are advised to start with the T-Streak, a three-phase streaking pattern. A sterile implement, such as a cotton swab or, more often, an inoculation loop, is used to apply the streaks. First, a flame is used to sterilise the inoculation loop. The loop is dipped into an inoculum, such as a broth or patient specimen, that contains a variety of bacterial species while it

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is cold. After that, the inoculation loop is moved in a zigzag pattern over the agar surface until around 30% of the plate has been coated. The plate is then rotated 90 degrees while the loop is re-sterilized. The loop is pulled across the area that had previously been streaked two to three times to continue the zigzag pattern. The process is then carried out one again, taking careful not to touch the areas that had previously been streaked. The loop collects less and fewer germs each time until it only collects a single bacterial cell that can develop into a colony. The initial area of the plate ought to have the most growth. The last portion will have the least development and the greatest number of isolated colonies, whereas the second section will have less growth and just a few isolated colonies. This is the isolation method that is most often used. In order to complete the procedure, a suitable sterile medium must be added to a sterile petri dish and allowed to set up. The agar surface is streaked with a very little amount of growth, preferably from a bacterial suspension or broth culture, until about one-third of the plate's circle has been covered. A sterilised looped, straight, or curved glass rod is used for this.

Each millilitre of the solution contains less and fewer germs when a variety of microorganisms are diluted. A very little amount of the dilution is then placed on a sterile petriplate using a sterile pipette or loop. The melted agar medium is poured onto a plate and allowed to cool to around 45°C. The bacterium and agar work nicely together. The specific bacteria will be preserved and grow into a clearly visible colony after the agar has dried. This method is used to isolate bacteria that are difficult to plate or streak. When many species are present in a mixture but only one dominates, this approach may sometimes be used to isolate the dominant form of an organism. For instance, a mixture of microorganism with a high percentage of Streptococcus lactis will be present at the time of curdling when raw milk is allowed to sour at room temperature.

Spreading the Plate and Pouring the Plate

Both the spread-plate and pour-plate procedures dilute a sample of cells before spatially separating them. The spread plate distributes the cells throughout the agar's surface, while the pour plate embeds the cells deep inside the substance. If known volumes of dilutions are plated, both procedures may be used to count the microorganisms in a sample. Why is dilution necessary? Dilution of the sample is necessary since it can include a lot of microorganisms. If bacterial colonies are plated without being diluted, there will be an excessive number of them and they will overlap, making it impossible to get an isolated colony. As a result, a little quantity of the sample is diluted by adding it to saline, phosphate buffer, or another suitable diluent. Dilute the sample many times. Both the spread plate technique and the pour plate method may be used to plate these dilutions. They both have benefits and drawbacks. Compare the two now.

Spread Plate: To create a spread plate, a little amount of a diluted mixture is placed in the centre of an agar plate and spread out uniformly using a sterile bent rod known as a spreader. The scattered cells form separate colonies as they grow.

Advantages

1. Only pick able, surface-level colonies emerge.

2. Heat-sensitive bacteria can also be isolated and the germs are not subjected to 450 °C.

The inability to separate microaerophilic organisms due to the development of only surface colonies.

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Pour plate: There are two methods to use these techniques. Agar that has been melted and chilled to roughly 45°C is combined with small amounts of numerous diluted samples, and the resulting mixes are then promptly placed onto sterile petri plates. In the second procedure, molten medium (450C) is poured on top of a little quantity of each dilution in the centre of an empty, sterile petri plate, stirred, and then allowed to solidify.

Planting

Each milliliter of the solution contains less and fewer germs when a variety of microorganisms are diluted. A very little amount of the dilution is then placed on a sterile petriplate using a sterile pipette or loop. The melted agar medium is poured onto a plate and allowed to cool to around 45°C. The bacterium and agar work nicely together. The specific bacteria will be preserved and grow into a clearly visible colony after the agar has dried.

Dilution

Measuring microbial counts for both liquid and solid specimens, such as E. coli suspensions in nutritious broth, soil samples, and hamburger, is standard procedure. The majority of samples include an overwhelming amount of microorganisms, making further dilution necessary for precise measurement. A step-by-step method for resolving dilution difficulties is provided below, followed by some practise issues. To count the amount of bacteria, fungi, or viruses may be the goal. This approach is specific for bacterial counts (CFUs), but it may also be used to fungus and virus counts (CFUs) (plaque-forming units, PFUs for viral counts). This method is used to isolate bacteria that are difficult to plate or streak. When many species are present in a mixture but only one dominates, this approach may sometimes be used to isolate the dominant form of an organism. For instance, a mixture of microorganism with a high percentage of Streptococcus lactis will be present at the time of curdling when raw milk is allowed to sour at room temperature.

According to the name, it is a series of successive dilutions used to turn a highly concentrated or dense solution into a less concentrated or high density one. To reduce a dense solution to a more useable concentration, a series of consecutive dilution stages are used in serial dilution. Simply said, serial dilution is a laboratory procedure that involves performing a stepwise dilution process on a solution with a corresponding dilution factor. This technique is used in the lab to reduce the numbers of viable cells in a culture in order to streamline the process. In a serial dilution, the serial number rises in each step as the cell count or density steadily falls. By computing the overall dilution across the whole series, it becomes simpler to determine the cell numbers in the main solution.

Serial dilution does not separate bacteria or viable cells like other methods like flow cytometry; it merely lowers the quantity of bacteria or viable cells. The basic goal of serial dilution is to gradually lower a substance's concentration, usually a chemical or biological sample. This method is used to precisely quantify the concentration of a material and to create solutions with a known concentration in various laboratory processes, including microbiology and biochemistry research. It may also be used to separate pure cultures of microorganisms from mixed populations and to estimate the exact number of bacteria or other microorganisms present in a sample. The technique for counting microorganisms, which was originally used to the study of water quality, is ascribed to Robert Koch. He wrote Detection Methods for Microorganisms in Water, which was published in 1883. A good approach for counting the quantity of bacteria and fungi is the standard plate count.

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Agar media is liquefied, a series of serial dilutions are made, and a sample of each is added before the medium is put onto a petri dish. The bacterial cells are contained inside the agar's matrix when it hardens. Within the agar, on its surface, and underneath it, colonies grow (between the agar and the lower dish). The procedure that follows produces a number of pour plates from various dilutions, although spread plates—samples that are spread on top of solidified agar—can also be used. It is feasible to count the microorganisms precisely because they are evenly distributed around the agar plate. This cannot be done in a fluid solution for two reasons: 1) it is impossible to assess the specimen's purity, and 2) it is impossible to count the cells in a liquid.

What does serial dilution serve?

The basic goal of the serial dilution method is to count the colonies that are cultivated from the sample's serial dilutions in order to determine the concentration or cell counts of an anonymous sample. Additionally, it utilised to save time by eliminating the need to pipette extremely tiny quantities (1–10 l) while diluting a solution. We can simply count the number of colonies that are produced on the incubated plates from the serial dilution, allowing us to count the viable cells that are present in the sample.

Enrichment

This procedure comprises utilising a medium and creating growing conditions that will help the selected species grow. For example, Salmonella typhosa is less common in the intestinal output of men with typhoid than E. coli and other strains.

Single Technique

One of the most effective and difficult methods for preserving pure culture is this. This method requires putting a suspension of the bacterial isolate atop a wet chamber on the microscope stage, behind a sterile cover glass. When viewing a single cell under a microscope, a sterile micropipette is used to remove it, transfer it to a small drop of sterile medium on a sterile cover glass, set it on a sterile hang drip slide, and maintain the proper temperature after that. In the event that the single cell starts to multiply in this drop, a few of the cells are transferred into a tube containing sterile culture medium and placed in an incubator to produce a pure culture produced from the single cell.

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CHAPTER 7

Maintenance of Culture

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The viability and purity of a microorganism must be maintained by maintaining the pure culture free of contamination once it has been isolated and cultured in pure culture. Similar to this, a microbiology lab must keep quality control (QC) stocks that were purchased from the ATCC or commercial suppliers. Testing culture medium, kits, and reagents need QC strains. To ensure that microorganisms may continue to develop and be viable, pure cultures are often subculturing—regularly moving them onto or onto a new media. To prevent contamination, the transfer is always submitted to aseptic conditions. It becomes challenging to sustain a large number of pure cultures effectively for an extended period of time because frequent subculturing takes time. Additionally, there is a chance of contamination and genetic alterations. As a result, certain contemporary techniques that do not need repeated subculturing are now replacing it. These techniques include lyophilization, cryopreservation, the paraffin process, and refrigeration (freeze-drying).

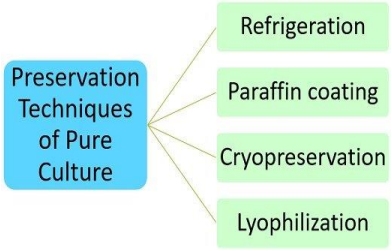

Figure 7.1: Prevention techniques of pure culture

Temporary Maintenance and Periodic Switch to New Media

By occasionally creating a new culture from the preceding stock, strains may be kept alive. It is necessary to determine in advance the culture medium, storage temperature, and interval at which the transfers are done. So that the period between transfers is as long as feasible, the temperature and medium should encourage moderate development rather than fast growth. On a media like nutritional agar, many more common heterotrophs may survive for many weeks or months. The transfer method's drawback is that it cannot stop strain characteristics from changing as a result of the emergence of variations and mutants.

Refrigeration

Pure cultures may be effectively kept in freezers or cold rooms between 0 and 4 degrees Celsius. Because the metabolic processes of the microorganisms are greatly slowed down but not halted, this approach is only used for a brief period of time (2–3 weeks for bacteria and 3–4 months for

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fungus). They consume nutrients, develop slowly, and emit waste materials into the medium as a result. After some time, the bacteria eventually perish as a consequence of this.

Glycerol preservation at -20 °C

On an adequate solid media, cultivate a pure culture. Scrape the culture off with a loop after it has reached its complete development. Small clusters of the culture should be suspended in sterile neutral glycerol. Distribute in vials or tubes with screw-on caps in amounts of 1-2 ml. Keep at -20 °C. Do not repeatedly freeze and thaw. After 12 to 18 months, transfer.

Cultures Stab

Staphylococci and Enterobacteriaceae are the only non-fastidious organisms that are employed in stab cultures at room temperature. Prepare tubes by adding a deep butt of agar devoid of carbohydrates. The use of tryptic soy agar is advised. the organism into the agar with a fork. Incubate at 35 °C all night. Use a cork or screw cap to seal the tube. To seal, dip a cap or cork into hot paraffin wax. Store in a warm environment. After a year, transfer.

Long-Term Preservation

Using long-term preservation techniques, subcultures may be separated by months or even years. If isolates are kept frozen at -70°C or below, they may be kept for an unlimited amount of time. These temperatures can be reached in a "ultralow freezer" (-70°C) or a liquid nitrogen freezer (-196°C). The best way to preserve bacterial cultures for a long time is by lyophilization or storage at -70°C or below; ordinary storage of isolates at -20°C is not advised.

Cooling to -70 °C

Aerobes and anaerobes may be kept for a long time by freezing at -70°C. Fastidious organisms should be thawed, reisolated, and refrozen every three years whereas frozen, non-fastidious organisms should be done so every five years. In 7H9 broth containing glycerol, acid-fast bacilli (AFB) may also be frozen at -70°C. In a solution containing a cryoprotectant, such as 10% dimethyl sulfoxide (DMSO) or foetal bovine serum, viruses may be kept at -70°C forever.

Cryopreservation

Pure cultures may survive for extended periods of time in storage because to cryopreservation, which involves freezing in liquid nitrogen at -196°C or in the gas phase above liquid nitrogen at -150°C. This technique involves quickly freezing cultured microorganisms in liquid nitrogen at -196°C in the presence of stabilising substances like glycerol or dimethyl sulfoxide (DMSO), which minimise cell damage from ice crystal formation and encourage cell survival. The majority of species may survive at these circumstances for 10 to 30 years without experiencing a change in their traits, and this liquid nitrogen approach has proven effective with many species that cannot be kept by lyophilization; nevertheless, this method is costly.

Cells, tissues, or organs are frozen during the cryopreservation or cryoconservation procedure in order to preserve the material for a long time. Any cell metabolism that can harm the biological material in issue is successfully halted at low temperatures (usually 80 °C (112 °F) or 196 °C (321) °F using liquid nitrogen). Long-distance biological sample transportation, long-term sample storage, and the creation of a sample bank are all made possible through cryopreservation. Cryoprotective agents (CPAs) are molecules that are added to the freezing process to lessen the

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osmotic shock and physical stressors that cells experience. Trees, wood frogs, and tardigrades are a few examples of plants and animals in nature with special cold tolerance that are utilised as inspiration for certain cryoprotective compounds in research.

Microscopic multicellular creatures called tardigrades can withstand freezing by displacing the majority of their internal water with the sugar trehalose, which prevents it from crystallising and harming cell membranes. Solute mixtures may have results that are comparable. Salts, for example, have the drawback of potentially being poisonous at high quantities. Wood frogs, like the water-bear, can withstand having their blood and other tissues frozen. In order to survive the winter, organs store urea, and liver glycogen is heavily converted to glucose in reaction to internal ice development. Urea and glucose both functions as "cryoprotectants" to prevent cell osmotic shrinkage and the quantity of ice that develops. Frogs can withstand several freeze-thaw cycles during the winter as long as just approximately 65% of their body water freezes. Dr. Kenneth B. Storey, a researcher from Canada, has particularly investigated the phenomena of "frozen frogs."

Few vertebrates, including five species of frogs (Rana sylvatica, Pseudacris triseriata, Hyla crucifer, Hyla versicolor, and Hyla chrysoscelis), one species of salamander (Salamandrella keyserlingii), one species of snake (Thamnophis sirtalis), and three species of turtles, have been found to exhibit freeze tolerance (Chrysemys picta, Terrapene carolina, Terrapene ornata). However, it has not been shown that it is adaptive for overwintering. Wall lizards Podarcis muralis and snapping turtles Chelydra serpentina also withstand little freezing. One cryopreservant in the case of Rana sylvatica is common glucose, whose content rises by around 19 mmol/L when the frogs are chilled gradually.

History

James Lovelock was a pioneering cryopreservation theorist. He postulated in 1953 that osmotic stress caused red blood cell damage during freezing and that harm may result from raising the salt content in a dehydrated cell. He experimented with cryopreserving rodents in the middle of the 1950s and found that hamsters could be frozen with 60% of the water in their brains crystallised into ice without suffering any negative consequences, however other organs were revealed to be vulnerable to harm. Three births brought about by the insemination of previously frozen sperm marked the commencement of the application of cryopreservation to human materials in 1954. A group of scientists working under the direction of Christopher Polge in the UK cryopreserved avian sperm in 1957. At Oak Ridge National Laboratory in the United States, Peter Mazur showed in 1963 that deadly intracellular freezing could be avoided if cooling was slow enough to allow for enough water to exit the cell during increasing freezing of the extracellular fluid. A common cooling pace of roughly 1 °C/minute is suitable for many mammalian cells following treatment with cryoprotectants like glycerol or dimethyl sulphoxide, although the rate is not a universally ideal one. This rate varies amongst cells of different sizes and water permeabilities.

Although it had been embalmed for two months, the first human corpse was frozen on April 22, 1966, by being immersed in liquid nitrogen and kept at a temperature slightly above freezing. The elderly Los Angeles resident, whose identity has not been released, was shortly defrosted and buried by family members. James Bedford, whose cancer-related death occurred in 1967, was the first person whose corpse was preserved in the hopes of being resurrected in the future. Only Bedford, who was frozen before 1974, is still in perfect condition. Storage of cells at very low temperatures is thought to extend their useful lives indefinitely, albeit the validity of this claim is difficult to establish. When scientists worked with dried seeds, they discovered that samples held

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at various temperatures, even very cold ones, varied noticeably in how quickly they deteriorated. It appears that biological activity very substantially slows down at temperatures below the glass transition point (Tg) of polyol's water solutions, or around 136 °C (137 K; 213 °F), and that 196 °C (77 K; 321 °F), the boiling point of liquid nitrogen, is the preferred temperature for storing important specimens. While many things can be stored in refrigerators, freezers, and extra-cold freezers, more complex biological structures are often preserved by liquid nitrogen's ultra-cold temperatures, which essentially cease all biological activity.

Lyophilization (Freeze-Drying)

After lyophilization, most organisms may be effectively kept (freeze-drying). Water and other solvents are eliminated from a frozen product by sublimation during the freeze-drying process. When a frozen liquid instantly transforms into a gas without first becoming liquid, this process is known as sublimation. Stock culture upkeep must be done correctly in order to do quality control, validate methods, and conduct research. Subculturing several times could ultimately result in contamination, viability loss, and genotypic/phenotypic alterations. Although cryogenic storage and freeze-drying are desired, they may not be feasible for smaller facilities. Regular laboratories may easily and affordably maintain a stock culture collection using cryoprotectant beads.

Lyophilization, or freeze-drying bacteria, is a fairly well-known technique for long-term preservation. The first accounts of freeze-drying bacteria date back to the middle of the previous century. While the methods employed differ greatly, they all adhere to the basic steps of lyophilization, which include freezing the sample, applying a high vacuum, warming the sample while under vacuum to cause water sublimation, removing excess water through a drying phase, and sealing the sample to prevent water absorption. Proteins, nucleic acids, yeasts, bacteria, fungus, and any other molecules that can be harmed by the presence of water are all preserved using this method in general.

There are four important factors to take into account while freeze drying microorganisms. The initial step is to prepare the cells by cultivating them. This is generally the same as how bacteria are routinely cultured. The second step is suspending the bacteria in an appropriate medium for freeze drying. Traditional alternatives like skim milk or sugar have been replaced by commercial formulations and those created by the ATCC. The freeze-drying procedure itself is the third factor to consider. This heavily depends on the kind of freeze drier being used and how many samples need to be preserved.

Making a Freeze-Drying Medium Selection

Bacteria must be suspended in a medium that supports their viability during freezing, water removal, and subsequent storage if they are to be preserved via lyophilization. The ideal remedy will include a substance that aids in forming a solid "cake," which, after being freeze dried, adds body to the bacterial suspension. This typically contains mannitol, skim milk, and bovine serum albumin as components (BSA). A lyoprotectant, which aids in maintaining the structure of biomolecules throughout the lyophilization process, is a second element of a suitable medium. Sucrose, a glucose that is joined to fructose by its C1 (anomeric) carbon, is the traditional lyoprotectant utilised with bacteria. Trehalose, a related compound (glucose connected C1 to C1 with another glucose), is often used to freeze-dry proteins but may be less successful in keeping bacteria alive. Salts are one element that shouldn't be utilised in freeze drying medium since they

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will concentrate during water sublimation and cause severe localised dehydration, which may kill cells.

To get excellent results, a few simple freeze-drying techniques are often utilised. A fairly common medium is skimming milk at a concentration of 20%, however viability after processing may drop by up to 90% or more (depending upon the strain). However, plenty of viable cells are still present if the sample's initial composition was more than 109 cells. As an alternative, a 5–10% sucrose solution is a common medium for freeze drying. Cake formation with sucrose is not as excellent as it is with solutions containing mannitol or BSA, but the sugar's capacity to shield the lyosomes results in high vitality. The American Type Culture Collection offers many options (ATCC). They have released a number of formulas for freeze drying solutions, including Reagent 18 (sucrose and BSA), which results in samples that freeze dry well and are maintained. Based on the Reagent 18 formulation, OPS Diagnostics created the Microbial Freeze-Drying Buffer, however instead of using BSA, they used plant protein.

The ideal cells for freeze drying are ones that have been collected and suspended in a medium for freeze drying. Typically, cells are grown in liquid media and then harvested by centrifugation. Alternately, cells may be removed by washing an agar plate that has just been streaked. As many strains may instantly suffer a considerable loss in viability as a consequence of the freeze drying, it is recommended to suspend and freeze dry at high cell densities, approximately 109/ml, in both situations. The culture will preserve at least some viable cells after extensive preservation if larger cell concentrations are used. The long-term viability of such preparations should be carefully examined. It is also feasible to simply inoculate cells into a freeze drying medium and lyophilize at extremely low cell densities.

Bacteria should be freeze dried in glass ampoules or vials. Water may really spread through various polymers over time, thus plastic should never be utilised. The design of the freeze dryer may also affect the kind of glass vessel that is utilised. A straightforward freeze-drying setup might be as simple as a high efficiency vacuum pump coupled to a cold moisture trap that is then directly linked to the sample. Long neck, heat-sealable ampoules need to be utilised in these circumstances. In the ampoules, dried bacteria are vacuum-sealed using a propane or acetylene flame. Although labor-intensive, flame sealing is the most secure way to keep the samples safe. Samples may be lyophilized in serum vials and sealed with rubber stoppers if the freeze dryer contains a drying chamber, as is the case with shelf dryers (called bungs). In order to enable water to escape during drying, these stoppers often include a split or notched base that is fitted into the vial aperture. Many shelf freeze dryers include a stoppering mechanism that forces stoppers into the vials, sealing them while they are under vacuum.

Glass tubes may be used by curators of cultural collections in place of vials or ampoules. To maintain culture collections, a variety of intricate (but effective) arrangements are utilised, such as sealed tubes put in larger tubes with labels and desiccant, which are then flame sealed shut. These methods are not covered in this article; instead, readers are advised to refer to guides like the ATCC Freeze Drying Handbook. Whether using ampoules or vials, they should only be filled up to around one-third of their whole capacity. Since smaller amounts are acceptable and speed up the freeze-drying process, putting 250 l of cell suspension into a 3 ml vial generates a large surface area relative to the volume, which will enable quicker freeze-drying processing. After being filled, ampoules are sealed with sterile cotton or glass wool to guard against sample contamination. After inserting bungs into vials, the samples are prepared for processing.

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Freeze-Drying Method

Three steps make up a standard freeze-drying procedure: freezing, primary drying, and secondary drying. There are numerous intricate variants on this fundamental procedure, but a simple method works well for the majority of bacteria.

Freezing

It is simple to freeze microorganisms before vacuuming sublime water by placing a prepared ampoule in a dry ice/ethanol bath. Rapid freezing preserves cell viability well, although it makes it more difficult to remove water. Water leaps off the ice and into the headspace above the sample when the frozen culture is put in a vacuum. Water naturally evaporates from a culture's surface first, then from the sample's core. Small pores or channels must develop to allow water to escape from the sample's interior before it may "sublimate." Channel development is often reduced during rapid freezing (with dry ice baths or even liquid nitrogen). Therefore, samples that have been promptly frozen need longer drying durations. By putting a rack of vials or ampoules in an ultralow freezer and allowing the culture to cool more gradually, samples may be frozen more gradually. Cultures may also be progressively frozen using the programmed temperature control that is often used in shelf dryers. Larger ice crystals grow in the sample at a slower rate of chilling, thus forming the channels for water evaporation. Although various bacterial strains may respond differently, it is normally beneficial to cool prepared cells from room temperature to -40°C over the course of 30 to 60 minutes. Test the freezing phase first, however, before committing to freeze drying several samples.

Initial Drying

The vacuum may be used after the bacteria samples have been frozen. Only high efficiency vacuums, or pumps with a pressure reduction capability of < 200 mtorr, can successfully freeze dry samples. The sample must be heated to a temperature greater than that of the cold trap in order to undergo primary drying. In simple systems, the cold trap is often a flask submerged in an ethanol and dry ice bath. Ampoules linked to basic systems start off chilly (the dry ice batch has a temperature of -70°C), but as they absorb ambient heat, they warm up. As long as there is a vacuum, the heat generates enough molecular motion to enable water molecules to sublime, or change from solid ice to gas. The secret to using high efficiency vacuums is to remove water more quickly than the sample warms up. The bacterial solution remains frozen as a result of the water's sublimation. The solution will dissolve if the sample's temperature rises too quickly, defeating the purpose of freeze drying.

In shelf freeze dryers, the condenser is chilled and acts as a cold trap. The shelf's temperature is likewise managed by the condenser. The shelf temperature is increased for primary drying such that the sample's water sublimes but does not melt. In this configuration, the shelf temperature may be adjusted to -10°C while the condenser maintains a temperature of -50°C. It is crucial that the sample doesn't melt when the water transitions from the warmer site (the sample) to the cooler place (the condenser). When creating a frozen sample that keeps its shape when the water is removed, the use of matrix-forming chemicals like BSA or mannitol is highly helpful. The sample would dissolve if the additives weren't there. Small sample sizes with high surface areas, as those with 0.5 ml in a 3 ml vial, enable water to be removed from them more quickly during this first drying step. For a small sample, primary drying may take 3–4 hours, but for a fully loaded shelf freeze drier, it can take all night. Empirical research should be used to establish how long is needed.

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Additional Drying

Bacterial culture drying is accomplished in two steps. Primary drying, as previously mentioned, eliminates easily accessible frozen water. Secondary drying raises the sample's temperature, which drives out any remaining water. The samples may be heated to 20°C for several hours in shelf dryers before being stoppered. It's crucial to avoid overdrying the germs since doing so might be harmful. For the same reason, using higher temperatures is not advised. A simple freeze-drying method does not enable the separation of primary and secondary drying. The sample's temperature will increase to reach ambient when the frozen water is forced from it. This ambient drying interval will act as a second drying step. Vials and ampoules must both be sealed after secondary drying. Press the stoppers into the vials when the suction is at its highest setting for shelf dryers with a stoppering mechanism. An acetylene or propane torch is used to seal ampoules by heating the long neck of the ampoule. The suction will assist in closing the glass. Use indelible ink to label all vials and ampoules so they won't rub or wash off. Be warned that samples may become ambiguous if labels come off.

Storage after Lyophilization

If no moisture enters the sample, freeze dried proteins may be kept at relatively warm temperatures. Not the case with bacteria. Long-term storage of bacteria at temperatures over 4°C will significantly reduce the vitality of the cells. At ambient temperature, bacteria that would typically last for years if stored in a refrigerator might quickly expire. Therefore, accelerated shelf life tests that simulate long-term storage by holding the sample at 37°C will not be successful with lyophilized bacteria. Vials and ampoules should be stored at 4°C for long-term storage. Periodically remove a vial or ampoule to count the remaining viable cells. It is important to monitor decay rates because they may be used to predict when a sample has to be revived and then freeze dried.

Preservation of Stock Culture

Maintaining a collection of bacterial and fungal stock cultures is one of the most crucial yet sometimes overlooked chores in any normal microbiology lab. The stock culture collection may quickly degenerate in a busy lab into a mess of incorrectly labelled, half dried-out agar slant cultures in the back of a refrigerator. However, it doesn't have to be that way and shouldn't be. A microbiology lab needs stock cultures in excellent health for a variety of reasons.

Techniques for Creating and Keeping a Culture Collection

It is crucial that the stock culture collection in the laboratory be adequately maintained, regardless of its size. In the past, this process included cultivating isolates on agar slants containing the appropriate medium, followed by subculturing onto new slants at regular intervals. The subcultures are kept in a refrigerator until needed or until the next subculturing is scheduled after incubation at an appropriate temperature. This approach has several significant flaws even if it works to a degree.

Contamination risk: Important isolates may eventually become fully overrun by contaminants.

Loss of viability: Sensitive isolates may become unrecoverable and lose viability if subculturing is not done as needed and cultures are not kept properly.

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Continued growth at cold temperatures: Some organisms, including Listeria monocytogenes, may continue to develop slowly in temperatures as low as 0°C. Labeling errors subculturing a large number of agar slants repeatedly increases the likelihood of incorrectly labelling a culture. Every subculture has the potential for genotypic and phenotypic alterations, such as the loss of virulence and resistance components or decreased motility. Genetic drift and mutation are two such changes. It is evident from this that stock cultures may pose major issues in the lab if they are not meticulously managed, and even a well-kept collection may eventually provide false findings. The American Type Culture Collection (ATCC) really advises against making more than five passes (subcultures) from the initial type strain, with the first passage being the culture produced from the ATCC-supplied vial.

Alternative approaches thankfully, labs are no longer restricted to using the conventional agar slant stock culture, and different techniques are available to simplify the collection maintenance procedure and get over the issues mentioned above. Freeze-drying, sometimes referred to as lyophilization, is a technique that may be used to stabilise bacterial and fungal cultures for long-term preservation by putting the metabolism of such cultures on hold. In order to create a thick suspension of bacterial cells or fungal spores, a suitable suspending media, such as 10% skim milk, or a particular lyophilization buffer must first be prepared. Then, the frozen suspension is poured into tiny glass vials. To remove water from the frozen condition, the plugged or loosely sealed vials are put in a freeze dryer's drying chamber and dried under vacuum for two to twenty four hours. It is also possible to apply a second drying stage by fastening the vials to the freeze-manifold dryer's for an additional 2 to 12 hours. Once the vial has finished drying, it is sealed and kept in the dark at a temperature of no more than 8°C.

Under these circumstances, many bacterial and fungal species will continue to be stable and viable for at least a year, and in some cases, cultures have been successfully revived several years afterwards. Some exacting or sensitive species, nevertheless, can be harmed by the procedure or perhaps go extinct. For instance, Campylobacter spp. cells and moulds that generate big, delicate spores need to be handled carefully since they are easily damaged and can only be kept for a limited amount of time.

For large commercial and government culture collections, freeze-drying is the go-to approach for preserving reference strains and other isolates for extended durations. However, it is also available to purchase select QC strains in simple-to-open containers that include hydration medium and an inoculating swab to facilitate resuscitation and subculture. Many culture collections provide freeze-dried cultures to labs in glass vials. For smaller labs that just need to preserve a modest stock culture collection, freeze-drying is usually not the best option. The method takes a long time, requiring a professional crew to verify that there is no contamination, and requires expensive freeze-drying equipment.

Cryogenic Storage often involves the use of liquid nitrogen, is an alternate way to keep cultures in environments that halt metabolism. To minimise damage during freezing, suspensions of bacterial cells or fungal spores are made in a cryoprotectant solution that typically contains 10-15% glycerol. The suspension is then poured into appropriate containers, such tiny screw-capped vials, and submerged in liquid nitrogen or suspended above it. Liquid nitrogen has a temperature of -196oC, which is far lower than the point at which all metabolic activity is assumed to stop. Not all microbial cultures will survive the technique, but those that do may live for a long time. This is similar to freeze-drying. In fact, certain isolates may have higher vitality than freeze-dried cultures.

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Cryogenic storage, however, is costly, requires a sizable volume of liquid nitrogen, and is probably best suited for bigger reference labs with substantial culture collections. Storage of frozen goods on cryoprotective beads Freezing cultures onto porous beads that enable cells and spores to cling to their surface offers some degree of protection against damage during freezing and may be the most practicable way of long-term microbial stock culture preservation for smaller facilities and those involved in regular testing. For this, labs may employ a variety of easily accessible commercial items, all of which function quite similarly.

Outline method

1. Create a suspension of the isolate's cells or spores, ideally from a culture that has been cultivated on solid media for 18 to 24 hours. In a cryogenic vial containing 20–30 of the porous beads, the colonial growth is suspended in a cryoprotectant solution, such as Brucella Broth with Glycerol.
2. The suspension is blended by inversion, and it is then let to stand for 15 to 20 minutes at room temperature. The porous beads, which have a high surface area to volume ratio, will develop a layer of cells during this time.
3. The surplus cryoprotectant liquid is then aspirated out, and the vial is immediately put into a freezer, ideally one that is no colder than -70oC. (Although the beads can be kept at -20oC, they will lose viability more quickly than at lower temperatures). To assist avoid a quick thawing when the cryogenic vials are taken out of the freezer, they may be stored in aluminium "cryoblocs."
4. Quickly extract a single bead and use it to inoculate a solid, non-selective medium to revive a culture. To prevent the remaining culture from thawing, the vial should be sealed once again and put back in the freezer right once.

When using this technique, stable stock cultures may be stored for a long time as long as the temperature is controlled at or below -70 ^{o}C. Additionally, the cultures are less susceptible to contamination. Since no costly or specialised equipment is needed, the approach may be used in small, ordinary labs. Additionally, the use of porous beads lessens the need for regular replacement and enables scientists to make greater use of freeze-dried reference cultures collected from significant collections. Any stock culture collection must be properly labelled in order to be kept in good condition. In order to aid labs in identifying certain kinds of cultures, cryogenic vials are also available with color-coded caps that may be labelled with permanent marker pens. In cases where stock cultures are employed for quality control and validation, accredited labs are often required to maintain records on the upkeep of such cultures. For this, specialised laboratory software programmes and simple computer spreadsheets may also be utilised, albeit it may be helpful to produce particular documentation. All stock cultures should have every subculture recorded so that the collection may be kept in excellent shape and updated as needed. Outsourcing allowing a specialised reference laboratory to assist with collection maintenance may be beneficial for labs with large stock culture collections, which may include rare strains isolated from patients, the environment, or damaged goods. Many commercial culture collections will keep critical cultures for customers and provide specialised freeze-drying services. This might be crucial if anything like a broken freezer compromises the integrity of the laboratory collection.

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CHAPTER 8

Staining Techniques

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Generally employed at the microscopic level, staining is a method for enhancing contrast in material. In the medical disciplines of histopathology, haematology, and cytopathology, which concentrate on the study and diagnosis of diseases at the microscopic level, stains and dyes are frequently used in histology (the microscopic study of biological tissues), cytology (the microscopic study of cells), and these other disciplines. Stains may be used to categorise distinct blood cell types, biological tissues (such as muscle fibres or connective tissue), cell populations, or organelles inside individual cells. In biochemistry, it is mixing a substrate with a dye from a certain class (DNA, proteins, lipids, carbohydrates) in order to identify or measure the presence of a particular component. Fluorescent tagging and staining have related uses. Additionally, biological labelling is employed in gel electrophoresis to identify proteins or nucleic acids and to mark cells in flow cytometry. The use of bright-field or epi-fluorescence illumination is usual when using light microscopes to observe stained materials at high magnification. The biological specimen is prepared. The kind of staining determines how the biological material is prepared for microscopy analysis. Several of the procedures are listed below. Dry Mounting Using water and certain dyes, living biological specimens are mounted on a glass slide. Fixation is a multi-step procedure used to maintain the structure of tissues and cells. The specimens are killed and adhered using heat fixation. Chemical fixation is used to create solid connections and boost the samples' stiffness. The most often used chemical fixatives include ethanol, methanol, picric acid, and formaldehyde.

Mordants: Otherwise, unstainable specimens are made stainable by adding mordants, which are chemical agents, to dye. There are two kinds of mordants:

Basic Mordants: These cause acidic dyes to react.

Acidic Mordants: These cause basic colours to react.

Indirect staining is the process of staining with the use of mordants. Direct staining, on the other hand, is the process of staining without the use of mordants.

Permeabilization: This process includes applying a surfactant to the specimen to breakdown the cell membrane and facilitate the easy staining of the sample with the dye.

Microorganisms must be stained in order to be seen under a light microscope since their cytoplasm is often transparent. When a drop of the microorganisms is put directly on the slide and viewed, staining is not essential in certain situations, such as when the bacteria are extremely big or when

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motility is being investigated. This kind of preparation is known as a moist mount. You can also create a wet mount by adding a drop of culture to a cover slip, a glass slide cover, and inverting it over a slide that has been hollowed out. The hanging drop is the name of this process.

A tiny quantity of microorganisms is put on a slide and allowed to air dry before staining. It is swiftly passed over a flame to quickly repair the smear using heat. The organisms are destroyed by heat fixing, which also causes them to stick to the slide and enable them to take the stain. Simple staining procedures e.g. basic dyes like crystal violet or methylene blue, which are positively charged dyes that are drawn to the negatively charged components of the microbial cytoplasm, may be used for staining. The straightforward stain process is one such method. Use of an acidic, negatively charged dye, such as Congo red or nigrosin, provides an option. The negatively charged cytoplasm repels them, causing them to cluster around the cells, leaving them clean and undamaged. The negative stain technique is the name given to this method.

In staining methods primarily two types of organisms may be distinguished using the differential stain method. The Gram stain method is one instance. Gram-positive and Gram-negative bacteria are divided into two categories using this differential approach. The stain is fixed by first applying crystal violet, then mordant iodine (Figure). The crystal violet iodine stain is then removed from the slide by washing it with alcohol, however it is retained by the Gram-positive bacteria while being removed from the Gram-negative bacteria. The counterstain, safranin dye, is then used to stain the Gram-negative bacteria. Gram-negative bacteria show blue or purple in the oil immersion lens, reflecting the crystal violet that was preserved throughout the washing process, whereas these bacteria look red.

The acid-fast method of differential staining is an additional method. Using this method, Mycobacterium species may be distinguished from other bacterial species. The first dye, carbolfuchsin, is introduced into the cells either by heat or a lipid solvent. After that, a diluted acid-alcohol solution is used to wash the cells. The carbolfuchsin stain is retained by Mycobacterium species even when they are resistant to the effects of acid-alcohol (bright red). Other bacteria absorb the following methylene blue stain after losing the original stain (blue). Thus, when seen under oil-immersion microscopy, the acid-fast bacteria are bright red and the nonacid-fast bacteria are blue. Other staining methods aim to pinpoint certain significant bacterial structures. For instance, a unique staining method accentuates the breadth of bacterial flagella by covering them with colours or metals. The flagella are then visible after being dyed. Bacterial spores are examined using a specific staining procedure. In order to drive the stain into the cells and give them colour, malachite green is heated. The non-sporeforming bacteria are subsequently coloured using the counterstain safranin. Sporae and other cells dye red at the conclusion of the operation.

Preparation for Smear

Most bacteria are tiny and transparent, making it difficult to see them under a microscope without preliminary staining. Before you can colour your germs, you must securely adhere them to a glass slide. When preparing a slide for staining, two crucial factors should be taken into account. The germs must be spread out softly and uniformly. You won't be able to examine the morphology of the individual cells if there are too many bacteria on the slide because they will group together and create a large glob. Large clumps of cells do not stain well either, which might lead to inaccurate

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findings. To prevent them from being washed away during the staining processes, the bacteria must be securely adhered to the slide. All techniques used to adhere the bacterium to the slide cause certain morphological modifications. The cells often demonstrate certain changes in form and extracellular matrixes, as well as a reduction in size. Slides will be set up for stains from both agar and broth surfaces. Although the end results for both are the same—equally and hardly scattered cells that are securely attached to the slide surface—the methods used are significantly different. Staining is both a science and an art. You'll probably need to try a few times before you succeed.

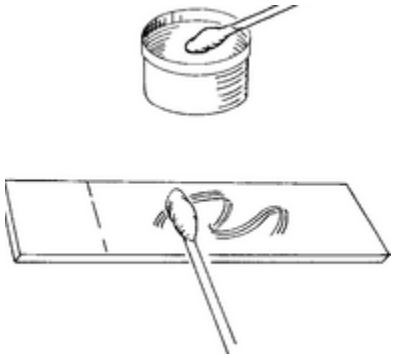

Figure 8.1: Smear Preparation

When moving bacteria from your loop to the slide, be mindful of aerosols. Because of the loop's extreme flexibility, it is simple to zing off a whole loop of creatures. Remember that your organism is not dead. It is not certain that the organism will be killed by heat or methanol fixation. Place your finished slides in the bucket of antiseptic at your workstation. You want to achieve a thin cell suspension that will leave a light, hazy deposit on your slide. Use the space on your slide; it's enough! To find your smear, it might be helpful to first draw a circle on the bottom of the slide. It is incredibly simple to mistakenly believe that your smear is on the wrong side of the slide. Make care to identify the slide's outermost edge. To help orient your slide, repeat this consistently on one end of the slide. Be patient and give your slide enough time to air dry before continuing to adhere it on the slide. The bacteria will boil and the cellular shape will be destroyed if you heat fix a wet slide. If you repair a wet slide in methanol, the fix will probably wash off the slide. Regardless of the fixation technique, excessively thick smears will certainly wash off the slide.

From Broth, Smear

Because the cells are already diluted in the broth, broth cultures are often simpler to deal with. To suspend the bacteria in the broth, properly mix the culture tube. Label the slide. Transfer an organism-filled loop aseptically to the middle of your slide. To spread the broth drop over the slide, use the flat portion of the loop. Spread out the drip by moving in a swirling, circular manner. Because the broth is so protein-rich, the smear often spreads out rather than congealing on the slide's surface.

Smudge on Plate

A lot of creatures may be removed using your loop. You could choose to transfer your organism to the slide using an inoculating needle. You dilute your samples, be sure to use sterile water. The

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deionized water in your rinse bottles or regular tap water is often contaminated with microorganisms.

- Label the slide. Transfer sterile water into the middle of the slide aseptically, one loop at a time.
- Your bacteria will be diluted as a result, and you will also have something to distribute.
- Select a colony that is well-off.
- Use your sterile needle to prick it, or use your sterile loop to gently scoop up the colony's edge.
- Put your needle or loop in the middle of the drop, then disseminate the germs around the slide in a spiraling, circular manner.
- Place the slide aside so it can air dry. This will take at least a few minutes. Do not skip this phase.

Fixation

No matter the source of the smear, the plate, or the broth, the fixing process is the same. Your bacteria may be adhered to the slide using either methanol fixation or heat fixation. BSL1 organisms are the only ones that utilise heat fixing. We will be dealing with BSL2 species, thus you must employ the methanol fixation method. With BSL2 organisms, we need to avoid aerosols as much as possible while heat-fixing the slide. The cellular morphology is not significantly altered by methanol fixation, and no aerosols are produced. If you don't remember that you repaired it with methanol and your slide isn't completely dry, the residual methanol will catch fire. Please use caution while dealing with the methanol. Simply put one stain to a fixed smear slide, let it sit, rinse it off, let it to dry, and then observe. Finding the presence and shape of bacteria in clinical samples like stools and discharges is a simple process.

To identify the morphology of spirochetes and fusiform in oral infections, utilise methylene blue. The preferred stain for recognizing the metachromatic granules in Corynebacterium diphtheriae is also this one. The granules will show a noticeably darker blue stain than the blue bacteria in the vicinity. There are no metachromatic granules in other Corynebacterium species. Any basic dyes, such as safranin, malachite green, crystal violet, or methylene blue, perform well. Both science and art go into staining. For staining and rinsing times, there are no absolute requirements. The suggested timings are guidelines that often function effectively. You'll need to test several approaches to see what works best for your particular bacterium. You must be very specific about what you do and the outcomes you see in your lab book. You don't want to spend time trying to reinvent your effective staining technique since you will be repeating these stains later in the semester. Each person working at the lab bench should use a different stain so you can compare how they all appear.

Simple Staining Method

- Set your meticulously created fixed smear slides on the stain rack over the sink.
- Work on one slide at once.
- Use any of the basic colours you have access to to cover the smudge.

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- Just enough colour is required to cover the smear. The slide shouldn't become wet with the stain.
- Let the spot sit for one to five minutes.
- Grab the slide's long end with the clothespin, slant it over the sink, and use the wash bottle's spray of water to rinse the stain away.
- Spray the solution above the smear and let it drip downward.
- You run the risk of washing the smear off the slide if you spray directly on it.
- Rinse until the water is either clear or hardly tinted.
- To get rid of extra water, touch the slide's edge to a paper towel. Now you may let it dry naturally. Alternately, you may softly press it dry with blotting paper by putting it in the blotting paper book. Even while this procedure is speedier, you may also wipe off a stain that has low adhesion.
- View your slide while submerged in oil, then note your findings in your lab notebook. Put your used, discoloured slides in the sink's disinfection bucket.

Negative Stain because you do not need to create a smear, negative stains are much easier to deal with than basic stains. Without fixation, cells are spread out on a slide and examined. India ink or nigrosin include a carbon suspension that causes the stain. The cell membrane and the carbon atoms both have negative charges. Since the cells repelled the dye, the backdrop appears black or sepia-toned while the cells are left clean. Some inclusion bodies with positive charges, like sulphur, may stain. Because the cells are not fixed, this dye provides precise information on cell shape and capsule existence. Due to the lack of staining, any extracellular coatings or secretions on the cell membranes outside give the appearance that the cell is significantly bigger. Negative stains may be used to quickly determine if Cryptococcus neformans, the cause of cryptococcisis, is present in cerebral spinal fluid. Additionally, this method is used while staining for endospores and capsules.

You just need a little bit of the organism, just as when making a smear. You won't be able to examine the shape of individual cells if there are too many creatures present. Additionally, it's crucial not to utilise too much nigrosin. The backdrop will have a cracked effect if it is too thick, like mud puddles drying in the sun. You wish to purchase a thin film. This method will be shown to you by your tutor.

Positive Staining Technique

Label the slide. Place a loop of organisms approximately three-fourths of the way up the left side of the slide if you are working from a broth culture. If you are working from a plate culture, place your organism in a drop of sterile water on the slide without spreading the drop. Put a drop or two of nigrosin on a different slide. Pick up a loop-full of nigrosin with your sterile loop. Without spreading the drop of cells too much, carefully combine it with the drop of cells. While holding the right end of the slide in your right hand, take another slide with your left hand, just beyond your nigrosin/cell drop, at a 45° or less angle to the previous slide. Slide the angled slide back down the first slide's surface until it just touches the nigrosin drop and cells. Watch for the liquid to be drawn down the angled slide's leading edge by capillary action.

Cross the flat slide's surface with the angled slide by pushing it. Still, the majority of the nigrosin need to be left in its original location. Place the slide in the bucket with the disinfectant. Before

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viewing the stained slide with oil immersion, let it aside to air dry. Make sure you begin looking at your slide where the grey backdrop is the palest. In your lab notebook, note your observations. Place your used, soiled slide in the bucket with the disinfectant. Nigrosin is incredibly simple to remove off the slide and transfer to your oil immersion lens. When you're through, be sure to properly wipe your oil lens. Then clean it one more. You won't be able to see properly out of the lens (as well as the other micro students using that scope) after it dries on the lens. It will also be extremely tough to remove.

Ink Stain

The most used differential stain in microbiology is the Gram stain. Differential stains use several dyes. How the bacteria respond to the various colours will depend on their particular cellular makeup. Since it was created in 1884, the Gram stain method has essentially not altered. Gram positive and Gram-negative bacteria may be used to categorise almost all microorganisms. Some bacteria have different gramme sizes. A Gram response may be seen in the cysts of Trichomonas, Strongyloides, certain fungi, and some protozoa. Treponema, Mycoplasma, Chlamydia, and Rickettsia are examples of bacteria without a cell wall or very minute bacteria that do not exhibit a gramme response. Any novel bacterium must have its gramme reaction described in their description.

The main stain, a mordant that sets the stain, a decolorizing agent to take out the primary stain, and a counter stain are the typical four components of a differential stain. Crystal violet serves as the main stain in the Gram stain. The cell now has a vibrant purple hue. Iodine, the mordant, and crystal violet combine within the cell wall to produce a complex. The cell is subsequently rinsed with either 95% ethanol or Gram's de-colorizer. Gram-positive cells will keep their purple colour and the dye complex. In gram-negative cells, the colour rinses off. The cells that lost the primary stain are coloured with the counter stain, safranin, otherwise they would stay colourless and you wouldn't be able to see them. Because of the chemical structure of the several layers of peptidoglycan in the cell wall, the massive iodine-crystal violet complex is kept inside the cell walls of gramme positive cells. Teichoic acids are extensively cross-linked, making it impossible for the iodine-dye combination to physically escape. The membrane has less lipid as a result, and the decolorizing chemical cannot reach it. Gram-negative cells only contain one layer of peptidoglycan, which is higher in lipid, and an outer membrane. The crystal violet dye may be removed with ease.

The strength of the bacterial cell wall determines how accurate the Gram stain will be. Old cells (i.e. cultures older than 24 hours), samples from people who have taken antibiotics that target that cell wall, such penicillin, harsh handling of the cells, or overheating the cells are a few examples of factors that might affect the integrity of the cell wall. Gram-positive cells will appear as gram-negative under these circumstances. Gram-positive cells will resemble Gram-negative cells if the decolorization process is prolonged. On the other hand, Gram-negatives will resemble Gram-positives if you do not decolorize enough. You must always run a known Gram-positive and a known Gram-negative on the same slide if you want to be able to trust your findings. If they stain as expected, you may be quite certain that the outcome of your unidentified sample is accurate. To master the Gram staining, practise is necessary. You shouldn't count on getting a decent Gram

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stain the first time. Holding your slide with a clothespin is a smart idea since anything you touch, including your gloves, will get fairly trippy.

Gram Stain Method

- Give your slide a name. Prepare your smears on a slide with a Gram negative on the left, a Gram positive on the right, and your unknown in the centre.
- Don't forget to repair your slide with methanol!
- After placing your slide on the staining rack, apply crystal violet to the smears and let it sit for a minute.
- Don't use too much stain; just enough to completely cover the smear without dripping or running off the slide.
- Tip the slide to one side and pour the crystal violet out. Rinse clean the slide quickly with water from your wash bottle.
- Keep in mind that spraying directly on the streaks will wash them away.
- For a minute, completely cover the slide with iodine mordant.
- Tilt the slide, remove any excess iodine, and use Gram's de-colorizer to carefully remove colour until the sample barely starts to flow clear.
- This is the challenging part!
- To get rid of extra de-colorizer, tilt your slide over some paper towels.
- For a minute, completely cover your slide with the safranin.
- Tilt the slide to drain any extra safranin, then gently rinse it under water until the water runs clean.
- Allow the slide to air dry.
- Keep your eyes immersed in oil.
- Gram negative controls should be pink, whereas Gram positive controls should be purple.
- Place your used slide in the bucket with the disinfectant.

The Congo Red Capsule stain is a variation of the nigrosin negative stain that you may have previously performed. The backdrop is then coloured with acid fuchsin dye after the microorganisms absorb the congo red dye. Both colours are excluded by the capsule or slime

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layers, which are highly hydrated polymers. The bacterial cells will look pink, the backdrop will appear blue, and the capsules will be seen as clear haloes. Using antisera specific for that kind of capsule, several extremely pathogenic bacteria, such as pneumococci, Haemophilus influenzae, and meningococci, may be recognised clinically by their capsules. Methlyene blue is combined with the bacteria after they have been suspended in the antisera. When the bacteria are stained with antisera, they will first look blue with a transparent halo around them, and then they will be encircled by a thin blue line where the antisera have connected to the capsule.

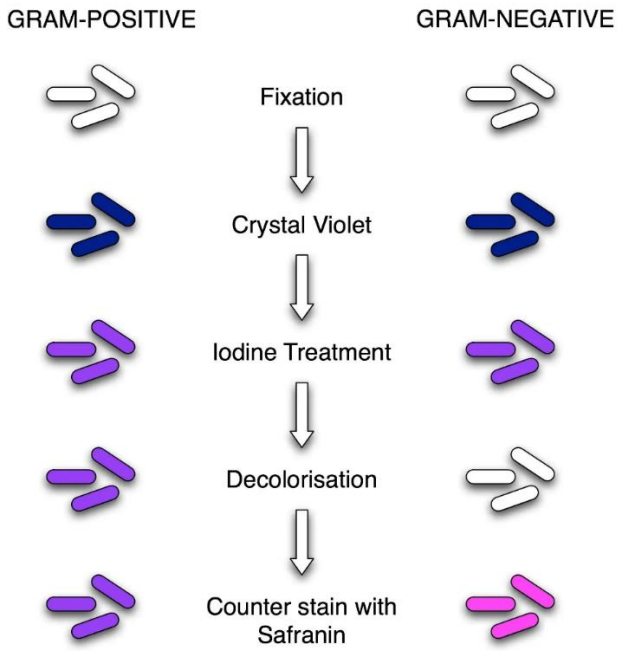

Figure 8.1: Gram Staining Method

The generation of endospores is exclusive to Clostridum and Bacillus species. They can withstand the unfavourable climatic conditions they encounter in their soil home because to their capacity to concentrate and cover their protoplasm. The spores may avoid discoloration as a result of this. Easy stains and Gram's stains make it simple to see the "living" organisms. Since endospores often have a high refractive index, light that strikes them is refracted. The inclusion bodies of several Bacillus species are quite refractile. If these inclusion bodies are stained normally, they may resemble endospores. Endospore-specific stains must be used to confirm the existence of endospores. Endospores' existence, as well as their distinctive location and form, need unique techniques to penetrate the endospore coat. The majority of endospore stains require heating the slides while continuously moistening them with the dye. Even while it is speedier, it creates volatile chemicals and is often a mess. If the dye is left on the slide for 30 minutes, the same results may be achieved. Starting this slide first is a smart idea since you may work on another stain while you wait for the dye to penetrate the endospore.

For the endospore stain, a Bacillus species will be used. B. cereus and B. anthracis spores resemble each other greatly in terms of location and morphology. Before it runs out of food, Bacillus does not begin to produce spores. The majority of what you will observe if the cultures are too young are the pink rods of the bacteria. You will mostly observe the endospores' little green ovals if the

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cultures are too old. The pink vegetative bacterial cell should ideally surround the green oval endospore bodies. For optimum results, choose a sample from the centre of a colony using the straight inoculating needle. The colony's edge is still expanding and will contain few endospores.

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CHAPTER 9

Techniques for Bacteria Cultivation

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An important component of the environment is bacteria. They play a significant part in food production, are essential to human health and the environment, and provide bioengineers the means to harness their capabilities and create compounds. But they may also be toxic, resulting in injury and illness. Therefore, being able to develop these bacteria is crucial to being able to harness their power, find dangerous offenders, and increase our knowledge and skills. In this article, we discuss the definition of bacterial culture, variables impacting the culture environment, typical issues, and some of the many uses.

Methods for Bacterial Culture

Bacteria need nutrients in the culture media in order to be properly cultivated. To meet the various nutritional requirements of bacterial species, there are several different formulations available. Your choice of media will depend on the goal of the culture. When attempting to bulk up a pure culture and get the bacterial cells in excellent condition, rich, nutrient-rich, or complete medium might be useful. On the other hand, minimal media will only provide the basic minimum for life and may be effective in controlling which bacterial pathways are activated. Furthermore, media may be categorised as defined or undefined. As the term implies, all the elements in a given medium are known. Complex combinations of nutrients and chemical species, like yeast extract, often appear in undefined media in undetermined amounts. Regardless of the medium used, the bacteria may be cultivated on a solid surface or in liquid form as a broth culture by adding agar to establish the media.

In contrast to static bacterial colonies, a culture in liquid medium, commonly referred to as a broth culture, allows the current bacteria free access to the available nutrients. This access may be further aided by gentle agitation to maintain the bacteria's dispersion throughout the medium throughout incubation. Liquid medium will also spread waste products throughout the culture through dilution as they develop. Therefore, compared to solid medium, a higher quantity of bacteria may be acquired for an equal amount of liquid media. Bacterial strains may be raised on liquid medium if long-term storage is the goal. The next step is to add glycerol, which will stop the bacteria from completely freezing and lysing as a result, allowing them to be stored at -80 °C. This kind of long-term storage keeps strains alive and is useful for long-term strain collections because it avoids the loss of important strains and lowers the possibility of mutations through repeated passaging.

In pathology labs all around the globe, broth cultures are regularly employed to help identify the kinds of bacteria causing a patient's illness. For many different sorts of medical practitioners, especially those handling urgent situations, it is an essential diagnostic tool. In research

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laboratories, broth media is also often utilised. It may be used in many different kinds of study to swiftly produce big numbers of a particular bacterial strain. Bacteria will often multiply exponentially if given all they need for success, giving the scientist quick access to test subjects. The bacteria may also be frozen for comparison with upcoming experimental generations or for usage in the future. Providing bacteria with a place to rest, heal, and proliferate after the colony has been stressed by an experimental source like heat or radiation is another purpose for them in the lab.

Depending on the tube's intended use, a variety of materials may be used to produce broth cultures. Lysogeny Broth (LB), created by Giuseppe Bertani in 1951, is a popular kind of broth. He created a fantastic medium that has several applications and contains sodium chloride, yeast extract, and tryptone. Sadly, it is sometimes misidentified as an abbreviation for Luria Broth, Luria-Bertani media, or Lennox Broth—neither of which is accurate. All of these terms are still often used in academia, despite the fact that the scientist himself dispelled this myth in 2004, and it's crucial to understand that they all refer to the same growth medium. There are many varieties of broth cultures, each with a distinct purpose. They are often divided into four categories, however. Only certain kinds of bacteria may thrive on selective medium, and all undesirable germs are killed. An example of this would be a scientist extracting harmful bacteria from a patient's sample of bacteria by purifying it, allowing it to develop, and then enabling it to be tested further.

Differential medium permits the development of all tested bacteria while also highlighting key variations in the kinds of bacteria present in the sample visually. Although it may be employed in broth form, differential media is most often utilised as solid or semi-solid growth medium. An example of this would be a blood agar plate that distinguishes between a bacterium's capacity for hemolysis (digestion of blood cells). In most cases, the bacteria will change colour or provide some other visual cue that will help the scientist identify the germs. Unspecified quantities of each component of the broth culture make up the media. All of the components are there, although in undetermined proportions. This often occurs in both types like LB, where yeast extracts provide a great growth environment but are immeasurable in terms of molecular makeup. Specific portions of each component of a broth culture make up defined media. These substances are often employed in investigations where the molecular composition must be determined and are typically compounds whose molecular weight and molarity can be precisely quantified.

Mineral agar

Liquid media may be established on petri plates as slopes or plugs, for example, by adding agar to it. When purifying a diagnostic sample, for instance, or when you want to choose certain colonies from a mixed culture, solid media might be helpful. Placing and incubation on solid medium also allow you to count the colony forming units (cfu) that are present in a certain volume of liquid sample. Another practical way to move strains from one lab to another without the risk of potentially infectious components spilling is by inoculating slopes or stab cultures.

A basic culture medium called Nutrient Agar is often used for the cultivation of non-fastidious microorganisms as well as for quality control and purity checks prior to biochemical or serological tests. By supplementing the medium with serum or blood, nutrient media may also be employed for the development of exacting microorganisms. For demonstration and educational purposes, Nutrient Agar is the best medium since it enables cultures to survive longer at room temperature without running the danger of overgrowth that may happen with more nutritional media. Standard techniques also advise using this medium, which has a very straightforward formula and is still

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often employed in the microbiological analysis of a range of materials. A general-purpose medium called nutrient agar is often used for regular culture or to assure the long-term survival of microorganisms. For the ordinary culture of microorganisms, it is one of the most significant and often used non-selective medium.

Numerous bacteria that are not very picky have been grown and counted on nutrient agar. By adding other biological fluids, such as horse or sheep blood, serum, egg yolk, etc., the medium may be made acceptable for the development of other finicky organisms. Numerous nutrients are used to make nutrition agar, which enables the development of a broad range of bacteria that ordinarily do not need a particular nutrient or supplement. The media's main ingredients are peptone, beef extract, and agar. Additionally, various vitamins and trace elements required for the development of bacteria are provided in addition to these nutrients.

The peptone serves as the bacteria's supply of nitrogen or protein as well as amino acids. The main source of carbon, which is necessary for the bacteria to produce carbohydrates, is the beef extract. Additionally, it includes additional elements that further promote the development of various species, such as certain vitamins, various trace minerals, salts, and organic compounds. Additionally, sodium chloride is supplied to the medium in order to preserve its osmotic balance and avoid a pH shift during growth. The nutrients are dispersed in the distilled water, making it simpler for the bacteria to consume them. The solidifying agent, agar, gives the organism a firm surface to develop on, enabling the study of colony form and the counting of the organism. The medium is a generic media that may be used for a number of applications since it contains the fundamental nutrients needed by a broad range of organisms.

Differential and Selective Media

There are also selective media that encourage or inhibit the development of certain species, groups of species, or strains with specified characteristics. This may be determined by a strain's capacity to use certain nutrients, its capacity to create particular byproducts, or its resistance to particular antibiotics. Selection is applicable to both solid and broth media. Color changes in differential medium may indicate a strain's capacity to thrive or lack thereof, and are often used to distinguish between various bacterial species or subtypes. For instance, with the use of analytical profile index (API) test strips, bacteria may be cultivated on a variety of substrates and identified by the different colour change patterns that they produce depending on their metabolism.

Antibiotics added to liquid medium will stop the development of non-resistant strains. When cultivating an engineered strain that has an antibiotic resistance gene introduced as a marker, this could be useful. Therefore, selection against the growth of contaminating species or colonies where the engineering has failed will occur. During preparation, antibiotics may be added to solid media, serving a similar purpose as liquid media. As an alternative, solid medium onto which a stain of interest has been inoculated may be covered with discs that have been injected with antibiotics. As the bacterial lawn expands around the disc where the strain is susceptible to the antibiotic, a distinct zone of no growth will become evident, allowing, for example, the selection of an appropriate antibiotic for the treatment of an illness.

The temperature and humidity ranges at which various species will develop ideally vary as well, reflecting the circumstances of oxygen and nutritional needs that have previously been mentioned and their native environment. Species that are often found deep inside the body, such as those in the gut or lower respiratory system, are probably best able to develop at body temperature, or 37

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°C. On the other hand, species that are located, for instance, in soil, probably need lower temperatures. Temperature may be used as a switch to regulate the integration of temperature-sensitive plasmids during genetic engineering of bacteria, therefore promoting the desired result.

Only certain bacteria are grown on selective medium. For instance, if a microbe is resistant to a certain antibiotic, such tetracycline or ampicillin, the antibiotic may be given to the medium to stop the growth of other cells that do not have the resistance. Before the advent of genomics, geneticists often employed media missing an amino acid, such as proline, together with E. coli incapable of synthesising it, to map bacterial chromosomes. In order to guarantee the survival or development of cells with certain characteristics, such as antibiotic resistance or the capacity to produce a specific metabolite, selective growth mediums are also utilised in cell culture. Normally, the capacity to develop in the selective medium is conferred to the cell by the presence of a certain gene or allele of a gene. The gene is known as a marker in certain circumstances. Neomycin is a typical component of selective growth medium for eukaryotic cells, and it is used to identify cells that have been transfected successfully with a plasmid containing the neomycin resistance gene as a marker. The exception to this rule is gancyclovir, which is used to precisely destroy cells that have the Herpes simplex virus thymidine kinase as their flag (HSV TK). Media that is selective includes:

Mannitol salt agar (MSA), which is differential for mannitol and selective for Gram-positive bacteria The "salt" in "Mannitol salt agar" favours bacteria that can flourish in conditions with a lot of salt. Staphylococci flourish in the environment, in large part as a result of their adaption to salty environments like human skin. Glycerol and Terrific Broth (TB) are both employed in the cultivation of recombinant Escherichia coli strains. Buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, including Legionella pneumophila. Xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria.

One kind of microbe may be distinguished from another growing on the same medium using differential or indicator media. The biochemical traits of a microbe growing in the presence of certain nutrients or indicators (such neutral red, phenol red, eosin y, or methylene blue) added to the medium are used in this kind of media to visually show the traits of a bacterium. This kind of medium is used by molecular biologists to identify recombinant bacterial strains as well as for the detection of microorganisms. Differential media examples include:

Blood agar, which includes bovine heart blood and becomes translucent in the presence of hemolytic, is used in strep testing.

- EMB, which differs for lactose and sucrose fermentation, is a streptococcus eosin.
- Differential for lactose fermentation is MacConkey (MCK).
- Mannitol salt agar (MSA), a special mannitol fermentation medium.
- Differential for lac operon mutations are X-gal plates.
- Acquisition of a pure culture.

The only bacteria present in a pure culture are those you want to grow. The source of your sample, the quantity of the target species relative to other species, and the target species itself are likely to have a significant impact on how easily this may be accomplished. The culture may already be pure if your source is another pure culture or a strain that has been separated and frozen. However, if the source is a clinical or environmental sample, there are probably many more bacterial species and maybe even fungi present, all of which will grow well under the conditions of your culture.

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Non-target species may be omitted, and the field can be made smaller, with the use of selective media and constrained growth conditions (such as aerobic vs. anerobic culture). Instead of placing the sample into a broth culture, spreading it over solid medium will enable you to visually distinguish the colonies of interest from the background. Before a pure culture can be established, it may be required to repeatedly pluck and re-streak bacterial colonies of interest onto new agar plates. Once this is accomplished, they may if desired be cultivated in liquid culture. Multiple plates from the initial sample may need to be streaked in order to isolate the target species if it is only sparingly present. It is also important to take into account the fact that certain species develop more quickly and fiercely than others.

Usages for bacterial culture

It may be essential or beneficial to cultivate bacterial cells for a variety of reasons. Here, we look at a few of the typical goals.

Identify the infection

Bacterial culture is still a vital diagnostic technique despite the time it might take to isolate and identify specific bacterial species from a sample. While PCR may quickly detect a particular virus, isolating the offender will prove that it is alive, warning researchers to possible transmission hazards and guiding treatment. Additionally, it allows for additional investigation of the bacterial strain to determine details like antibiotic sensitivity and guide treatment decisions. Additionally, strains may be preserved for later use, such as for disease monitoring.

Genetic engineering

For a variety of purposes, including attempting to understand the fundamental biology, attenuating it while developing vaccine strains, increasing protein production, and establishing a reference strain with a detectable marker, it may be advantageous to change the genome of bacterial strains. Before, during, and after the genetic engineering process, the target strain must be cultured, regardless of whether genetic material is being altered, deleted, or added.

Epidemic analysis

For epidemiological investigations, culturing and classifying bacterial strains might be crucial. In addition to studying transmission events, which may help influence things like public health policies and recommendations, this allows scientists to analyse how bacterial populations evolve over time. This can help with the creation and updating of therapeutics, vaccines, and diagnostics. One such initiative, the Gonococcal Isolate Surveillance Project (GISP), keeps track of strains for antibiotic resistance in order to assist guide recommendations for medication treatment. The Active Bacterial Core Monitoring (ABCs) system, which provides laboratory- and population-based surveillance of invasive bacterial pathogens of public health relevance, is also operated by the Centers for Disease Control and Prevention (CDC).

Increase scale to allow omics investigations

Next-generation sequencing (NGS) is still carried out on material from many bacterial cells for many studies, despite the fact that sequencing of DNA and RNA can be done with minuscule amounts of genetic material, even at the single-cell level. As a result, the bacteria frequently need to be cultured before DNA or RNA extraction. In contrast to microbiome research, which usually include a mix, if you are interested in a single strain, it will probably come from a pure culture.

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Develop medicines and vaccinations

A bacterial pathogen must normally be able to be cultured in order to be combated. It could be required to cultivate strains in order to comprehend their genomes, amplify their genes, or otherwise alter them during the production of vaccines16. Equally, challenge experiments17, in which subjects are exposed to the pathogen to see if the therapy is successful, are sometimes required to test out potential vaccinations or treatments. To do this, the bacterial strain is often grown and counted in a specified challenge model to regulate and establish the dose that participants receive.

Food and beverage production

Probiotics and starter cultures are two general categories for the bacteria that are used in the manufacturing of numerous meals. Probiotics are typically grown for their benefits to human health, often through our gut microbiome,18 and are widely used today. While there are many other bacterial species that may be found in probiotics, the most popular options for cultivation are Lactobacillus and Bifidobacterium. On the other hand, starter cultures are often utilised as a step in the food manufacturing process to generate taste, texture, nutritional value, or enhance preservation. Salami, pepperoni, and dried ham are a few examples. Sourdough breads are another. Starter cultures often include lactic acid bacteria (LAB). However, certain foods and beverages, including yoghurt, kimchi, which is becoming more and more popular, and kombucha, might perhaps fall into both categories since they are eaten for both taste and their probiotic properties. Keeping a culture healthy and free of contaminants is essential for optimum production and customer safety, regardless of the function for which it is designed.

Identifying food-borne toxins

Despite the fact that certain bacteria may be advantageous for the production of food, they may also be present as contaminants and have the potential to result in life-threatening foodborne diseases. Listeria monocytogenes, Campylobacter jejuni, Salmonella spp., and E. coli are typical culprits. The ability of analysts to cultivate any potentially dangerous bacteria from food samples is crucial, even if they are present in little amounts.

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CHAPTER 10

Techniques for Fungi Cultivation

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Important organisms for fundamental research, industry, and human health are filamentous fungus. The many techniques used for their isolation and cultivation mirror the wide range of their natural growing settings. Agar plates, shaking flasks, and bench-top fermenters are often used in the laboratory to cultivate fungi, using an inoculum that normally contains fungal spores as the first step. With a focus on enzyme production and molecular microbiology, we address the most widely used techniques for the isolation and development of filamentous fungus here.

Growing the fungus from a patient sample is the quickest and most reliable way to make the diagnosis of a fungal illness. Blood, cerebrospinal fluid, pus, urine, tissue, respiratory samples (sputum, bronchoscopy lavage), pleural, pericardial, or peritoneal fluid, skin scrapings, hair, nail clippings, oral, or vaginal samples are just a few of the many samples that might produce fungi. These samples may need to be processed by centrifugation or softening/liquidization in order to be spread out onto an agar media. The procedures for achieving this are described in several laboratory manuals and detailed guides, but surprisingly few comparison studies comparing one procedure with another have been carried out.

The significance of media choice

Direct cultivation of samples on 'fungal medium' improves the yield of the majority of fungi. Histoplasma, Mucorales, and Coccidioides spp. are a few fungus for which cultures on bacterial medium are consistently or virtually always negative. Approximately 30% fewer Aspergillus spp. may be grown on bacterial medium than on fungal media.

Sabouraud dextrose, malt extract, and less often brain heart infusion medium are general purpose media that are frequently used for fungi cultivation. Chloramphenicol is used to avoid bacterial contamination of the medium, however it inhibits Actinomyces growth, despite other bacteria growing well on Sabouraud dextrose agar. Cycloheximide is used to lower the frequency of environmental fungal growth, however doing so lowers the yield of several opportunistic fungus, such as isolates of Aspergillus species, Cryptococcus neoformans, and Mucorales. As a result, if cycloheximide is utilised, a separate agar plate should be used in parallel.

Media for presumptive identification and selective culture

Additional specialized media can be applied directly to specimens to enable the separation of colonies with similar appearances. Examples include the use of chromogenic agar for the direct, partial identification of Candida spp. or bird seed agar to identify Cryptococcus spp. in respiratory cultures from HIV patients.

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Fungus blood culture

Numerous blood culture systems, including "normal" bacterial cultures and more specialised ones like the Septi-Check systems, the lysis centrifugation systems, and specific bottles inside an automated system, have been utilised (sometimes of Mycobacteria and fungi). There have only been a few comparisons of these systems, and the decision becomes much more important when the list of possible infecting fungus includes Penicillium marneffei, Histoplasma capsulatum, and Cryptococcus neoformans. Both aerobic and anaerobic bottles should be filled with blood because Candida glabrata grows more slowly and often in the anaerobic bottle in modern automated blood culture systems. If patients are not taking antifungal medications [2] and at least 20 mL of blood is cultured, the yield from the test is greater. Conditions for incubation Cultures should be incubated for 21 days in a humid atmosphere at a temperature of 30 oC, which is the recommended incubation temperature for fungi. For at least a week, they should be checked daily, and then at least three times a week after that. Some fungi, including Histoplasma spp., which grow extremely slowly, may need longer incubation periods. Candida species cannot develop in respiratory cultures grown at 42 °C, enabling Aspergillus species to thrive unhindered.

Managing growth and identification methods

Once colonies are seen, their morphology should be closely examined. Yeasts may also be recognized by biochemical tests and by the way they grow on specialized medium like cornmeal agar. On chromogenic medium, yeast identity may be inferred based on colony color and shape. There are a few fast-testing techniques that are more or less complete and dependable. There are published comparisons of these approaches. Growing the fungus from a patient sample is the quickest and most reliable way to make the diagnosis of a fungal illness. Blood, cerebrospinal fluid, pus, urine, tissue, respiratory samples (sputum, bronchoscopy lavage), pleural, pericardial, or peritoneal fluid, skin scrapings, hair, nail clippings, oral, or vaginal samples are just a few of the many samples that might produce fungi. These samples may need to be processed by centrifugation or softening/liquidization in order to be spread out onto an agar media. The procedures for achieving this are described in several laboratory manuals and detailed guides, but surprisingly few comparison studies comparing one procedure with another have been carried out.

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It is more difficult to recognise filamentous fungus. The only meaningful differentiation that can be discerned in the absence of spores or other sterile structures is whether the hyphae are septate or not (i.e., whether they are Mucorales, Basidiobolus ranarum, or Conidiobolus spp. Included among the media that may help with sporulation is potato dextrose agar. If the colony won't spore, the only method of identification left is molecular. An expert mycologist can often identify a fungus to at least genus level once the colony has sporulated using phenol cotton blue or another straightforward method of emphasising the characteristic structures of the fungus. Without further information, such as colour and shape of other medium, differential temperature growth rates, and molecular data, species identification of filamentous fungus may be very difficult or impossible. Numerous cryptic (i.e., same in appearance) species from several genera have been reported.

MALDI-TOF

With matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, several fungus may now be speciated (MALDI-TOF). A portion of the colony from a successful culture is combined with a specific reagent and examined in the mass spectrometer right away. The time it takes to get the answer is around one minute. The MALDI-TOF mass spectrometer operates at a high level of quality thanks to the constant use of calibrators and controls. An integrated database uses the ionisation spectrum to offer identification. But few filamentous fungi, particularly Candida spp., are adequately represented in the commercial databases now in use. One of the difficulties is that the development patterns of filamentous fungus are often extremely varied,

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which means that factors such as medium, growth conditions, and temperature might affect protein spectra.

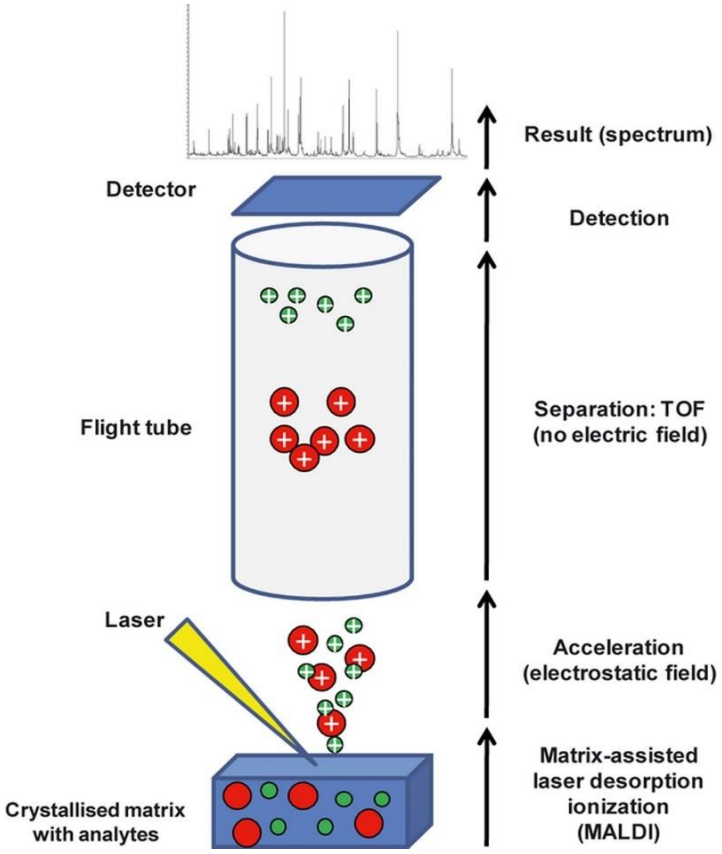

Figure 10.1: MALDI-TOF MS Method

Clinical microbiology laboratories may choose between the Bruker (Autoflex) and BioMérieux commercial systems (VITEK MS). They vary in terms of apparatus, databases, and organism identification algorithms. Each database is closed-access and private. Yeasts may be recognised both directly from blood cultures and via culture plates. When blood includes mixed Candida species or mixed bacterial/fungal cultures, MALD-TOF functions less effectively. For the Aspergillums genus as well as the genera Mucor, Rhizopus, and Lichtheimia of filamentous moulds. 82-97% of isolates have their species (complex) levels of identification valid. Dermatophytes are also quite well identified. Large studies are difficult to conduct because uncommon moulds are rare. MALDI-TOF MS reduces turnaround time for bacterial and yeast identification by an average of 1.5 days and does not need culturing compared to traditional procedures.

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CHAPTER 11

Techniques for Actinobacteria Cultivation

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Due to the fact that actinobacteria (actinomycetes) generate a wide range of naturally occurring medications and other bioactive compounds, they have drawn a lot of interest. Since actinomycetes (actinobacteria) generate a wide range of organic medicines and other bioactive metabolites, such as enzymes, inhibitors of certain enzymes, and antibiotics, they have drawn a lot of interest. In the scientific and patent literature, more than 22,000 bioactive secondary metabolites from microorganisms, including antibiotics, have been found and published. Actinomycetes are responsible for producing nearly half of these chemicals. A total of 160 antibiotics are now in use for medical purposes and in agriculture, and actinomycetes are the source of 100–120 of these drugs, including streptomycin, erythromycin, gentamicin, vancomycin, vermectin, etc. It is becoming harder and harder to employ common methods to create novel actinomycete-based medicines. Over 90% of all microbes are still difficult to cultivate, despite a significant number of them having been discovered, characterised, screened for, and utilised. The discovery of novel drugs may be made possible by these uncultivable bacteria. Selman A. Waksman continuously and methodically screened soil fungus and bacteria between 1914 and 1939 in an effort to discover a TB antibiotic. He learns in 1939 that some fungus, in particular actinomycetes, have an impact on the development of bacteria. He succeeded in 1944 with the development of Spectromycin thanks to his 1940 ability to isolate the potent T.B. antibiotic actinomycin. He received the Nobel prize in physiology and medicine in 1952 for all of his efforts.

The phylum Actinobacteria, which is one of the biggest taxonomic groupings of the 18 major lineages now recognised under the Domain Bacteria, includes filamentous Gram-positive bacteria known as actinomycetes. These bacteria have a complicated life cycle. Actinobacteria are extensively spread in terrestrial and aquatic habitats, mostly in soil, where they play a crucial part in recycling refractory biomaterials by breaking down intricate polymer combinations in dead plants, animals, and fungi. They play a significant role in the biodegradation of soil and the formation of humus because they recycle the nutrients associated with recalcitrant polymers like chitin, keratin, and lignocelluloses. This results in the production of several volatile substances, including geosmin, which is responsible for the distinctive "wet earth odour," as well as diverse physiological and metabolic characteristics, such as the production of extracellular enzymes. Actinomycetes, which create 10,000 of the 23,000 bioactive secondary metabolites that microorganisms make, account for 45% of all known bioactive microbial metabolites. The Streptomyces species among actinomycetes create over 7,600 different chemicals.

Powerful antibiotics may be found in a number of these secondary metabolites. Due to this, streptomycetes have replaced other species in the pharmaceutical sector as the main producers of

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antibiotics (Berdy 2005). This group includes companies that make clinically effective antitumor medications like anthracyclines (aclarubicin, daunomycin, and doxorubicin), peptides (bleomycin and actinomycin D), aureolic acids (mithramycin), enediynes (neocarzinostatin), antimetabolites (pentostatin), carzinophilin, mitomycins, etc. Nevertheless, one of the top priorities for cancer treatment continues to be the quest for new medications. The desire for innovative anticancer medications that are active against fewer adverse effects with incurable tumours and with higher therapeutic effectiveness has grown due to the fast development of resistance to several chemotherapeutic treatments and their unfavourable side effects. Through the use of high-throughput fermentation and screening, combinatorial biosynthesis, and genome mining for cryptic pathways, new secondary metabolites related to existing pharmacophores have recently been produced in actinomycetes, advancing the field of drug discovery from these microorganisms. Since marine actinomycetes have been isolated from sediments all over the globe, including the deepest sediments from the Mariana Trench and the shallowest costal sediments, it is clear that actinomycetes are always present in marine sediments, although in less quantities than in soil.

Only 1% of the tiny living forms that make up marine microorganisms have been cultivated or identified, yet they consist of a complex and varied assemblage. Additionally, marine sponges in particular have been reported to live in symbiosis with marine actinomycetes. Marine actinomycetes have drawn a lot of attention because they have evolved physiological and metabolic abilities that not only ensure their survival in harsh environments but also provide the potential to produce compounds with antitumor and other interesting pharmacological activities that would not be seen in terrestrial microorganisms, possibly as a result of their close ties to marine eukaryotic organisms like mammals.

Actinobacteria may be isolated using this fundamental premise. Three goals generally are pursued while isolating actinomycetes. The first research focuses on the actinomycete community in a unique setting. It is necessary to isolate and identify each and every actinomycete in this situation as pure cultures. The isolation medium that is being employed has to be favourable to the development of more actinomycetes while inhibiting the growth of other microorganisms in order to get to this aim. It is advised to utilise three to five different mediums. In the medium, Gram-negative bacterial and fungal inhibitors should be introduced.

The second step is isolating unique actinomycetes, including those belonging to a recognised species or genus or those with unique physiological traits like tolerance to chemicals, alkalis, acids, salts, high and low temperatures, as well as antibiotics. Target actinomycetes should have all of their needs met by the isolation medium, whereas unwelcome microorganisms should have their proliferation inhibited. In order to separate halophytic and alkalophytic actinomycetes, for instance, the medium's pH level should be raised to 10 to 12, and the salt content of the isolation media should range from 15% to 25%.

The third is isolating unidentified actinomycetes. Numerous actinomycetes from different ecosystems throughout the globe have been isolated and identified up to this point. So, although it's challenging, isolating unidentified actinomycetes is crucial. Gram-negative bacteria, certain Gram-positive bacteria, fungus, and the majority of common actinomycetes must all be kept in check in order to do this. Test samples are gathered from many sources. In a variety of natural settings, such as soil, lakes, the ocean, plants, and animals, actinomycetes may be found as saprophytes. The discovery of new actinobacteria continues to be successful in soil. Primary

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ecological variables, including nutrient, aeration, pH, temperature, salinity, moisture, and organic matter content, have a significant impact on the amount and kind of actinobacteria present in soil and other substrates. It is true that the selection of environmental samples may have a significant impact on the effectiveness of isolating large numbers of a particular actinobacteria. It is ideal to take soil samples from virgin areas like ancient forests, saline soils, alkaline soils, and deserts. In a sterile paper or plastic bag, soil samples from a depth of 5 to 20 cm are placed.

Oceans include a variety of actinomycetes, and these organisms have yielded a great deal of natural goods. With the use of a sampler, sediment from the deep ocean is gathered. The samples are then placed in glass bottles that have been sanitised and kept at 4°C. Plants are home to many actinomycetes. Novel plant endophytic actinomycetes are also a possible source of antibacterial and anticancer drugs, notably from traditional Chinese medicines. Various plant tissues are taken as fresh samples and placed right away in sterile containers. In order to isolate actinomycetes as quickly as feasible, the fresh samples should be utilised.

Testing sample preparation

Actinomycetes, which grow more slowly than other bacteria and fungus, must undergo pretreatment in order to be isolated with precision. Generally speaking, pretreatment regimens choose target actinomycetes by preventing or getting rid of undesirable germs. The isolation of actinomycetes has used a variety of chemical and physical pretreatments. Since most bacteria are less resistant to desiccation than actinomycete spores, most unwanted Gram-negative bacteria can be removed from soil, sediment, lichen, and faecal samples by simply allowing them to air dry at room temperature. This prevents isolation plates from becoming overrun with these bacteria. To isolate certain actinobacterial taxa with precision, soil may be heated either air-dried or in suspension. In contrast to streptomycetes, the actinomycetes are referred to as uncommon actinomycetes. The uncommon actinomycetes have yielded a significant number of new bioactive compounds in recent years. As a result, isolation techniques for uncommon actinomycetes have attracted a lot of interest.

Actinobacterial culture

To research the bioactive compounds generated by actinomycetes, the culture is restricted to tiny liquid and solid fermentation. In the process of finding novel medication leads, fermentation is a crucial step. The components, concentration, pH, duration, temperature, and aeration of the broth are all factors that alter according on the strain. These are the fermentation's main objectives in general. To investigate the bioactive components of actinomycetes, employ the fermentation broths listed below. Choosing the best broth and fermentation durations requires fermenting each strain with 4 to 8 broths for 4 to 7 days.

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CHAPTER 12

Understanding the Microbial Growth

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Bacterial growth is a complicated process that includes various anabolic (cell components and metabolites are synthesized) and catabolic (cell constituents and metabolites are broken down) events. These biosynthetic processes ultimately lead to cell division. A cell may divide in as little as 10 minutes in a homogenous, rich culture medium when all factors are favorable. On the other hand, it has been proposed that in certain deep terrestrial habitats, cell division might happen as slowly as once per 100 years. Numerous variables, including the fact that the majority of subterranean ecosystems are diverse and nutrient-poor, contribute to this delayed development.

As a consequence, cells are more likely to be isolated, unable to exchange nutrition or defence systems, and unable to reach an efficient enough metabolic state to support exponential development. Controlled laboratory investigations employing pure cultures of microorganisms have produced the majority of the knowledge that is now accessible about the development of germs. Batch culture and continuous culture are two methods for studying development under these controlled circumstances. In a batch culture, the development of one organism or of a consortium of organisms is assessed using a specified medium to which a predetermined quantity of substrate (food) is first introduced. Continuous culture maintains the same quantity of substrate accessible due to a consistent inflow of growth media and substrate. Both medically and statistically, growth under batch and continuous culture conditions has been thoroughly documented. The commercial production of several microbial products, such as antibiotics, vitamins, amino acids, enzymes, yeast, vinegar, and alcoholic drinks, has been optimised using this knowledge. Large-scale fermentations, another name for the production of these compounds in bulk (up to 500,000 litres), is a common practise.

Microbes may expand rapidly when given the proper circumstances (food, the appropriate temperature, etc.). This might either be advantageous for humans (yeast growing in wort to produce beer) or detrimental depending on the circumstance (bacteria growing in your throat causing strep throat). Knowing how they grow will help us forecast or manage their development under certain circumstances. Microbial growth is evaluated by the increase in population, either by counting the number of cells or the rise in total mass, as opposed to multicellular development, which is normally quantified in terms of the growth of a single organism.

Division of the bacteria

Only asexual reproduction is possible in bacteria and archaea, however both sexual and asexual reproduction is possible in eukartyotic microorganisms. A single cell divides into two identically sized cells in a process known as binary fission, which is most often used by bacteria and archaea.

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Multiple fission, budding, and spore generation are among other, less frequent processes that may occur. The cell expands and has more cellular components before it divides. The circular chromosome's origin of replication, which is where the chromosome is connected to the inner cell membrane, is where DNA replication begins after that. Up to the termination, replication proceeds in opposing directions along the chromosome.

The expanded cell's centre shrinks until two daughter cells are created, each of which receives a division of the cytoplasm and a copy of the parental genome in whole (cytokinesis). A protein known as FtsZ controls this cytokinesis and cell division process. A Z ring formed by FtsZ forms on the cytoplasmic membrane (Figure 9.3). The Z ring, which marks the division plane between the two daughter cells, is attached by FtsZ-binding proteins. The Z ring is joined with other proteins necessary for cell division to create a structure known as the divisome. When the divisome is activated, it builds a septum that separates the two daughter cells and a peptidoglycan cell wall. The division septum, where all of the cells' outer layers (the cell wall and outer membranes, if present), must be remodelled in order to complete division, separates the daughter cells. For instance, we are aware that some enzymes may release the bonds holding the monomers of peptidoglycans together, allowing for the insertion of additional subunits along the division septum.

Cell elongation, the first step in the process, calls for a meticulous expansion of the cell wall and membrane in addition to an increase in cell volume. In order to have two copies of its chromosome, one for each freshly produced cell, the cell begins to duplicate its DNA. The septum, which first appears as a ring in the centre of the elongated cell, is formed with the help of the protein FtsZ. The septum is formed, splitting the elongated cell into two daughter cells of equal size, after the nucleoids are segregated to either end of the elongated cell. For an active culture of E. coli bacterium, the full procedure or cell cycle may be completed in as little as 20 minutes.

Phase-I: According to research done by Lutkenhaus and his colleagues on FtsZ-Receptor Binding, FtsZ localises to the location of future division early in the division cycle and stays connected to the invaginating septum throughout cytokinesis. The interaction of FtsZ with the cytoplasmic membrane at the future division site is a necessary early step in the series of events that leads to septation and cell separation because it acts earlier in the division process than the other known components of the division machinery, with the possible exception of ZipA (see below).

The site-specific membrane recognition event suggests that a particular FtsZ receptor is present at midcell. We haven't yet found the receptor, but we could be getting close to the prey. A direct search for genes encoding for proteins that interact with FtsZ produced ZipA, a newly discovered integral membrane protein, which was published in a recent issue of Cell. The data suggests that the ZipA-FtsZ contact occurs in the midcell location at a relatively early stage of the division cycle because ZipA has numerous characteristics that are anticipated of the FtsZ receptor. First, in vitro binding of FtsZ by ZipA, a membrane protein that is essential. Second, the production of nonseptate filaments in the absence of ZipA indicates that ZipA is necessary for cell division. Instead of the filaments linked to loss of other division genes that operate at later stages of the division cycle, the zipAnull filaments morphologically resemble ftsZnull filaments. Third, almost every cell in the expanding population has a ZipA-green fluorescent protein (GFP) fusion protein localised at midcell. Last but not least, ZipA-GFP is distributed at the division site in a pattern like that of FtsZ, with ZipA situated in a ring that circles the cell. These findings suggest that ZipA either interacts with FtsZ contemporaneous with or shortly after the first FtsZ-membrane contact,

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or that ZipA is the FtsZ receptor. The outcome of trials to determine whether or not FtsZ can localise to the midcell location in the absence of ZipA will be used to decide between these options.

Phase-II: Development of the FtsZ Ring

The FtsZ ring, which is connected to the inner surface of the cytoplasmic membrane and surrounds the cell, is created by the membrane-associated FtsZ in a process that most likely takes place soon after its first connection with the membrane. The polymerization of FtsZ protomers is thought to form the FtsZ ring. This is in line with the finding that FtsZ can be made to polymerize in the presence of GTP, which results in the creation of filamentous polymers with a protofilament structure resembling microtubule. There are between 10,000 and 20,000 FtsZ molecules per cell, which is enough to form a number of continuous polymeric structures around the circumference of the cell. FtsZ isolated from the soluble fraction of the cell exists as an oligomer whose gel filtration behaviour suggests a size of 10-15 monomers per oligomer. The ring may be composed of one or more polymers that extend completely around the cell, or it may be a series of shorter polymers organised to form a continuous annulus. This species is thought to be the one that binds to the receptor and initiates the polymerization of FtsZ.

The preseptal domain at midcell may have numerous sites for the initial binding of FtsZ, and multifocal polymerization may take place from each of these sites to account for the development of the final ring. Recent immunofluorescence studies on the spherical cells of rodA mutants have shown that FtsZ may sometimes be seen as a partial annulus that does not entirely encircle the cell. This suggested that FtsZ may first interact with one another or start polymerization on that side of the cylinder. The whole annular structure that is present in most cells would then be formed by extending the ring all the way around the cylinder. The first FtsZ-membrane contact could only need a limited number of receptors that are concentrated at one spot if the FtsZ pattern of the rodA mutant cells mirrors the pattern of ring formation in wild-type cells.

Phase-III:: Putting together the Cytokinesis Equipment

At least seven additional protein elements are necessary for cytokinesis in addition to FtsZ and ZipA. These comprise six integral cytoplasmic membrane proteins and the peripheral membrane protein FtsA. (FtsI, FtsQ, FtsL, FtsW, FtsN, and FtsK). For FtsA, there is concrete proof that the protein is positioned at the site of cell division. The evidence is still inferential for the others. With several hundred copies per cell compared to the majority of the other membrane-associated division proteins' less than 50 copies, FtsA stands out from the others due to both its abundance and apparent peripheral membrane attachment. In contrast to the membrane-associated FtsA, FtsA recovered in the soluble fraction of shattered cells is evidently phosphorylated (16). Since an unphosphorylated version of the protein is still capable of restoring cell division in an ftsAats mutant, it is not yet known if FtsA phosphorylation affects the assembly or function of FtsA.

According to immunofluorescence investigations and the analysis of a FtsA-GFP chimaera, FtsA is distributed in a circumferential ring at the site of division similarly to FtsZ. Since the FtsA ring was not seen when creation of the FtsZ ring was blocked by mutation of ftsZ or by production of the SulA (SfiA) division inhibitor, it is clear that this pattern of FtsA localization needs a functioning FtsZ protein. As anticipated, FtsA filaments did not stop FtsZ ring development. Studies with ftsZ26 mutant cells, in which a mutation in the FtsZ coding sequence leads in a spiral pattern of septum development and a concurrent spiral pattern of the FtsZ ring, have provided further evidence that the localization of FtsA to the division site is reliant on FtsZ. (2). The similar

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spiral distribution of FtsA was seen in these cells. As a result, for FtsA to be localised, it must directly interact with FtsZ or another division component whose localization is influenced by FtsZ. Unknown is whether FtsA relocates to the membrane concurrently with FtsZ or after FtsZ attaches at the location of cell division. The discovery that overexpression of either FtsA or FtsZ blocks division, and in each instance, the division block may be removed by concurrent overexpression of the other protein, suggests that FtsA and FtsZ interact. The necessity for a certain FtsZ/FtsA ratio for correct assembly or operation of the division apparatus is compatible with the direct interaction of the two proteins, even if it isn't direct evidence.

Transmembrane proteins (FtsI, FtsQ, L, W, K, and N; 4, 7, and 12) make up the other known division proteins. The fact that the FtsZ ring formed unaffected by the ftsAts, ftsIts, or ftsQts mutations suggests that the FtsZ ring is formed before to or independently of the assembly of these division apparatus components. Although the spiral pattern of septal ingrowth in the ftsZ26 strain (see above) suggests that the organisation of the entire division apparatus may be determined by the pattern of FtsZ organisation within the ring, it is unknown whether assembly of FtsI, FtsQ, FtsL, FtsW, FtsK, and FtsN into the division machinery depends on the prior assembly of the ftsZ ring. Bitopic proteins FtsI, FtsQ, FtsL, and FtsN only make one lipid barrier crossing. These proteins seem to engage in division-related processes that take place outside the cytoplasmic membrane since they each have a sizable periplasmic domain.

Murein production during cell elongation does not need the murein biosynthetic enzyme FtsI, but it is necessary for the creation of the murein layer of the division septum. Since the septal murein layer is located outside of the cytoplasmic membrane, FtsI's function is likely definitely to catalyse its formation there. The transmembrane domain and small cytoplasmic domain of FtsI are also necessary since they cannot be substituted by the comparable domains of the unrelated MalG inner membrane protein without losing function. This is true even though the periplasmic domain is crucial for FtsI function. It is unclear if FtsI's transmembrane and cytoplasmic domains are essential just for the protein's correct assembly into the division apparatus or whether they provide another, more directly related function.

By observing that chimeric FtsQ proteins in which the membrane-spanning and cytoplasmic domains of FtsQ were replaced by the corresponding regions of MalG were still capable of restoring division function to a temperature-sensitive ftsQ strain, domain-swapping studies of FtsQ have confirmed the essential role of its large periplasmic domain. Since a soluble periplasmic form of FtsN is sufficient to restore division activity to an ftsN null mutant, the function of FtsN also seems to be located there. Surprisingly, the division defect of certain ftsA and ftsI mutants is suppressed by overexpressing FtsN. As a result, it is possible that FtsN contributes to the assembly or stability of the septation machinery or perhaps to the indirect control of the expression of certain division genes.

Cytokinesis

The cytoplasmic membrane, the murein layer, and the outer membrane are all circumferentially ingrown as part of the intricate process of septal invagination. This process might be explained by two models. According to the PUSH model, a cell cycle signal is sent from the division apparatus to the machinery responsible for producing septal murein at the centre of the cell. The cytoplasmic membrane is then "pushed" inward by the ingrowth of the stiff murein layer at an angle to the long axis of the cell. According to the PULL model, cytokinesis is started by the FtsZ ring contracting, which "pulls" on the cytoplasmic membrane and sends a signal to the machinery responsible for

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generating septal murein to start the process. It is crucial because FtsZ homologs are found in bacteria like Archaea and Mycoplasma that do not contain murein when trying to differentiate between these models. This suggests that constriction of the FtsZ ring during cell division can occur independently of murein ingrowth, arguing against a straightforward PUSH model of bacterial cytokinesis. If the ftsZ gene products in these organisms are a component of their division apparatus, as one would suspect, this would be the case. Therefore, it is probable that the FtsZ ring's constriction is a crucial step in the creation of the septum, with septal murein ingrowth acting as a subsequent or even parallel process in response to the same signal that causes the ring to contract. The FtsZ Ring is constricting. According to immunoelectron microscopy, the FtsZ ring is missing from the progeny cells' newly formed poles but stays connected to the septum's leading edge throughout septal ingrowth. Therefore, by the time septal closure and cell separation take place, processes are needed to both produce ring contraction and remove FtsZ molecules from the septal location.

In order to explain these facts, we look at two hypotheses. According to one concept, the FtsZ ring would gradually shrink due to the gradual extrusion of individual FtsZ subunits into the cytoplasm and the subsequent reconstruction of the end-to-end connection of the remaining ring subunits. This model's linkage of the two known phenomena—contraction of the ring and expulsion of FtsZ molecules from the division site—makes it appealing. An alternative theory postulates that the FtsZ ring behaves like an array of microtubules or actin filaments, providing a track that is traversed by a motor protein that is responsible for the progressive shortening of the ring during cytokinesis. This theory is based on the similarity of FtsZ protofilaments formed in vitro to microtubule protofilaments (8). The motor may do this, for instance, by supplying the driving power for a sliding filament response in which the ring's segments slide past one another to gradually reduce its circumference, much like a muscular contraction.

The loss of FtsZ from the division site by the time of septation termination would thus need the use of an additional mechanism. Since FtsA has been shown to react with an ATP-agarose column and includes a predicted nucleotide-binding domain, indicating that its function may entail an energy-dependent step, it is a contender to be a part of the motor if this turns out to be the mechanism of ring contraction. Septal Murein growth. Using one or more of the other division proteins as signal transducers, a signal provided by the start of FtsZ ring contraction is passed on to FtsI and possibly other proteins involved in the synthesis of septal murein in a plausible scenario that links septal murein synthesis with other cytokinesis events. Given that it has been shown that an ftsAats mutant dramatically reduces the penicillin-binding activity of the putative septal murein synthetase, FtsI, FtsA may be implicated in the coupling process. invasion of the outer membrane It is understood that mutations that alter the attachment of the murein layer to the outer membrane may separate the ingrowth of the outer membrane from the ingrowth of the septum. These include deletions of the lpp gene and lkyD mutations, which prevent the covalent attachment of the murein layer to the Lpp lipoprotein of the outer membrane (previously called lpo). In both instances, the outside membrane is unable to invaginate properly into the developing septum, which suggests that the outer membrane is only a passive passenger being drawn inside by the formation of septal murein.

Bacteria are simple to cultivate in the lab, thus their growth has been widely researched. It has been established that bacteria will develop in a predictable manner in a closed system or batch culture (with no food added and no wastes removed), resulting in a growth curve with four distinct phases of development: the lag phase, the exponential or log phase, the stationary phase, and the

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death or decline phase. Additionally, this growth curve may provide an organism's generation time, or how long it will take for the population to double. Unfortunately, it is challenging to apply our understanding of development in tightly controlled laboratory settings to growth in natural soil or water environments, where more complicated degrees of growth are present. A variety of diverse solid surface types, microenvironments with varied physical and chemical characteristics, a restricted nutritional status, and consortia of various microbes all vying for the same scarce food supply are some of the reasons that contribute to this complexity. Understanding microbial development in natural habitats is therefore a current concern for environmental microbiologists. Predicting rates of nutrient cycling, microbial reactions to anthropogenic environmental perturbations, microbial interactions with organic and metal pollutants, and the survival and development of diseases in the environment would all be made easier. In this chapter, we explore pure culture growth first, and then we talk about how it relates to growth in the environment.

Typically, cells are put in a liquid medium with regulated nutrients and environmental conditions in order to comprehend and characterise the development of a specific microbial isolate. If the environment is ideal and the medium has all the nutrients needed for development, it is possible to quantify the rise in bacterial mass or numbers as a function of time to create a growth curve. Within a growth curve, several development stages may be seen. These consist of the death phase, the stationary phase, the exponential or log phase, and the lag phase. Every one of these stages denotes a unique stage of development that is connected to normal physiological changes in the cell culture. As you'll see in the sections that follow, each phase's growth rates are quite distinct from one another.

The Lag Period

The lag phase, in which the growth rate is basically nil, is the first phase seen under batch settings. After a period of time known as the lag phase, growth starts to occur when an inoculum is added to fresh media. After the original population has doubled, the lag phase is said to change into the exponential phase. The physiological adaptation of the cell to the culture conditions is assumed to be the cause of the lag phase. The induction of particular messenger RNA (mRNA) and protein production to satisfy new culture needs may take some time. The exoenzymes (enzymes secreted from the cell) and nutrients that leak from developing cells may be diluted as a consequence of low initial densities of organisms, which may also be the cause of the lag phase. These supplies are often shared by cells that are near to one another. However, these materials are diluted and less readily absorbed when cell density is low. The beginning of cell growth and division as well as the change from the exponential phase may be delayed as a consequence.

The lag period might last anywhere from a few minutes and many hours. Because it depends on the kind of media and the initial inoculum size, the duration of the lag phase may be somewhat adjusted. For instance, there won't be any lag phase if an inoculum is removed from an exponential phase culture in trypticase soy broth (TSB) and added to new TSB medium at a concentration of 10 6 cells/ml while maintaining the same growth parameters (temperature, shaking rate). However, there will be a lag period if the inoculum is obtained from a stationary phase culture as the stationary phase cells adapt to the new environment and physiologically transition from stationary phase cells to exponential phase cells. Similar to this, a lag phase will be seen when the cells reorganise and shift physiologically to generate the proper enzymes for glucose catabolism if the inoculum is put into a medium other than TSB, such as a mineral salts medium with glucose as the only carbon source. Last but not least, if one is monitoring activity, such as the removal of

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substrate, and the inoculum size is low, say 10 4 cells/ml, one will see a lag phase until the population reaches around 10 6 cells/ml. Figure 3.4, which compares the degradation of phenanthrene in cultures injected with 10 7 and 10 4 colony-forming units (CFU) per millilitre, serves as an illustration of this. The lag phase lasted 1.5 days when a low inoculum size was utilised (10 4 CFU/ml), as opposed to just 0.5 days when a larger inoculum (10 7 CFU/ml), even if the degradation rate attained is identical in both instances (see the slope of each curve).

Exponential Phase

The exponential phase is the second growth phase that may be seen in a batch system. The exponential phase is defined by a time of exponential growth, which is the fastest growth conceivable given the batch system's constraints. The rate of cell growth in a culture during exponential growth is related to the number of cells present at any given period. This idea may be expressed in a variety of theoretical and mathematical ways. One method is to think about how the number of cells rises during exponential growth in the geometric sequence 2 0, 2 1, 2 2, and 2 3 until, after n divisions, the number of cells is 2 n.

Stationary phase

The stationary phase is the third stage of growth. Cells continue to grow and divide in stationary phase even when there is no net growth. Simply put, an equal number of cells dying offsets growth. A batch culture may enter stationary phase for a number of reasons. One frequent cause is when a vital food or carbon source is fully depleted. When a carbon supply is depleted, growth does not always come to an end. This is so that lysing dying cells may serve as a source of nutrition. Endogenous metabolism is growth on dead cells. Although endogenous metabolism takes place during the whole growth cycle, it can best be seen during stationary phase, when growth is gauged by oxygen absorption or carbon dioxide evolution. The accumulation of waste products to a level where they start to hinder cell development or become harmful to cells is another factor that might lead to the observation of stationary phase. Only high cell density cultures often experience this. No matter why cells reach stationary phase, growth there is imbalanced because certain components are simpler for the cells to manufacture than others. Cells will continue to grow and divide as long as feasible even when certain components become more restrictive. Cells in the stationary phase are often smaller and rounder than cells in the exponential phase as a consequence of this nutritional stress.

When a cell is in its stationary phase, its rate of growth and death are equal. Both the buildup of hazardous substances and the depletion of nutrients in the medium restrict the pace of bacterial cell development. At this stage, the cell population is steady. This phase produces a smooth horizontal linear line when plotted on the graph. Several variables cause the growth rate to slow as the number of cells grows during the log phase. As nutrients are steadily depleted, waste products build up. Additionally, aerobic cell development starts to be restricted as oxygen levels gradually decrease. This confluence of negative factors causes population growth to gradually slow down and then stop. The stationary phase is characterised by a plateau in the total number of living cells (Figure 9.5). The overall population of live cells is essentially static at this stage since the number of new cells produced by cell division is now equal to the number of cells degenerating. In a stationary culture, the culture density remains constant. The carrying capacity of a culture, also known as the maximum culture density, depends on the types of microorganisms that are present in the culture as well as the environment in which those microorganisms are grown.

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Carrying capacity, however, is constant for an organism that is grown in a culture under the same conditions.

Cells change their metabolic state to one of survival during the stationary period. Since the production of peptidoglycans, proteins, and nucleic acids slows down along with growth, stationary cultures are less vulnerable to antibiotics that interfere with these processes. Many cells of bacteria that may produce endospores sporulate during the stationary phase. Antibiotics and other secondary metabolites are produced during the stationary phase. The expression of virulence factors, substances that help a germ survive, proliferate, and infect a host organism, is also linked to the stationary phase in certain dangerous bacteria. For instance, Staphylococcus aureus uses quorum sensing to start the synthesis of enzymes that may break down human tissue and cellular detritus, allowing germs to move to fresh tissue with more nutrients.

The Death Phase

The final phase of the growth curve is the death phase, which is characterized by a net loss of culturable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are

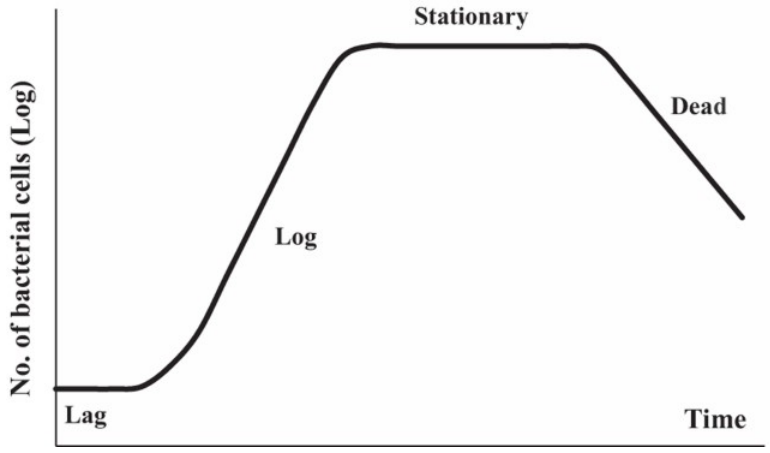

Figure 12.1: Microbial Growth Curve

This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells. Lack of nutrients, physical conditions or other injuries to the cell leads to death of the cells.

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CHAPTER 13

Factors Affecting Microbial Growth

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Creating and maintaining a safe and healthy work environment is essential for having a productive workforce. It's necessary to maintain tools for the job and a building's structural integrity, but there may also be dangers present that can't be seen by the human eye in a company's infrastructure. When possible, issue locations with elements impacting microbial growth aren't recognised and monitored on a regular basis, fungi and other bacteria often grow unrestrained in the crevices and crevices of a structure. Building-wide fungi and bacterial growth may get into the ventilation systems and make people sick and uncomfortable. It's crucial to understand what types of environmental conditions affect microbial growth and monitor areas of concern with the assistance of environmental consultants to keep air ventilation and water sources clean and free of health hazards given that conditions like Legionellosis, or Legionnaires' Disease, and chronic upper respiratory problems are potential and persistent issues that could be caused by microbial growth.

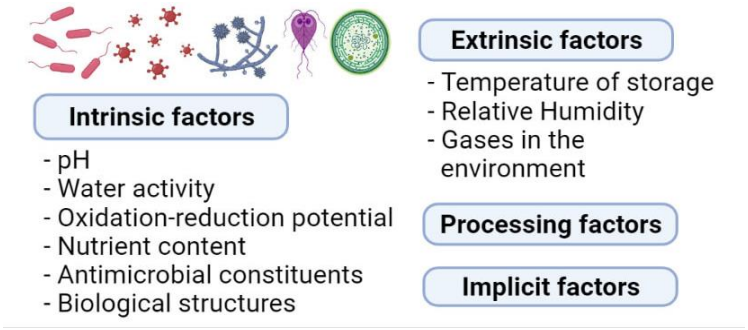

Figure 13.1: Factors affecting the growth of microorganisms in food

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 themselves 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.

Influences on Microbial Growth

Creating and maintaining a safe and healthy work environment is essential for having a productive workforce. It's necessary to maintain tools for the job and a building's structural integrity, but there

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may also be dangers present that can't be seen by the human eye in a company's infrastructure. When possible, issue locations with elements impacting microbial growth aren't recognised and monitored on a regular basis, fungi and other bacteria often grow unrestrained in the crevices and crevices of a structure. Building-wide fungi and bacterial growth may get into the ventilation systems and make people sick and uncomfortable. It is crucial to understand what types of environmental conditions affect microbial growth and monitor areas of concern with the assistance of environmental consultants in order to keep air ventilation and water sources clean and free of health hazards given that conditions like Legionellosis, or Legionnaires' Disease, and chronic upper respiratory problems are potential and persistent issues that could be caused by microbial growth.

Dampness and water

The four main physical and chemical parameters influencing microbial development are warmth, moisture, pH levels, and oxygen levels. The two main general problems in most structures are temperature and wetness. Moisture has a major role in the development of fungus. Like all living things, bacteria need water to survive. Without a reliable water supply nearby, they are unable to grow and spread. The likelihood of moisture and standing water in bathrooms and basements makes them prime locations for possible microbial problems. In addition to damaging equipment, leaks in the ceiling caused by rain or from neglected pipes in the water system may serve as breeding grounds for germs in places that are more difficult to detect or clean up.

Temperature

The temperature of a place may have a significant impact on the development of microorganisms. In warm environments, when the temperature is near to that of the human body, bacteria grow most rapidly. When food is chilled to make it safe to consume for a longer period of time, cooler environments tend to limit the development of microorganisms. Molds and germs may grow in boiler rooms, heat-generating equipment rooms, and spaces close to heating vents. Naturally, the spots that might raise the greatest worry when considering the health of a building are those near equipment that generates dampness. By developing solutions using techniques like optimising circulation with optimum air ventilation, an environmental consultant may assist you in preventing possible health risks before they ever arise by assessing regions surrounding equipment with heat as a consequence.

As a whole, microorganisms may thrive in a broad range of temperatures, from below freezing to above boiling. The temperature range across which growth is feasible for every organism is defined by its lowest and maximum growth temperatures; this is generally between 25 and 30 C. Low temperatures impede growth because enzymes function less effectively, lipids tend to solidify, and membrane fluidity is lost. Temperature-dependent growth rates rise until the optimal temperature is attained, after which they begin to decline (Figure 5.4). The temperature range of an organism's enzyme systems, which in turn are governed by their three-dimensional protein structures, is reflected in the optimal and limiting temperatures for that organism. The optimal temperature is often higher than the lowest and lower than the maximum growth temperature. Once the optimal value has been reached, the rate of growth abruptly declines due to the loss of activity brought on by enzyme denaturation.

Mesophylls are the majority of microorganisms, which thrive best at "middling" temperatures between 20 and 45 °C. Compare them to thermophiles, which have evolved to not only survive at

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greater temperatures, but to thrive in them. These could typically develop between 40 and 80 °C, with an ideal temperature of 50 to 65 °C. Extreme thermophiles can withstand temperatures of more than 100 C and have optimal values above this. A primitive bacterial species known as the Archaea was believed to have set a new world record in 2003 by thriving at 121 degrees Celsius. The other end of the temperature spectrum is occupied by psychrophiles, which may grow as low as 0°C but thrive best at temperatures of 15°C or below. At temperatures over 25 C or so, these creatures are unable to grow. Psychrotrophs, on the other hand, have substantially greater temperature optimal ranges (20-30 C), despite the fact that they may also develop at 0 C. Due of their capacity to thrive on chilled meals, members of this group are frequently economically important.

Ecological pH

Microbe development may be aided or hindered by the pH of an environment. Microbes often suffer when more basic or acidic substances are present in an environment because they prefer pH values that are neutral. Because of this, using cleaning solutions—which are often quite acidic—kills germs efficiently. This indicates that, when carried out frequently, enhancing cleaning procedures in a company's building may significantly lower the amount of germs developing and discourage future growths. By adding acid or alkali to growth medium as it is being prepared, the pH value is changed to the desired value. It is crucial in a laboratory growth medium that a desired pH be not only established but also maintained since the metabolic activities of microorganisms often cause them to adjust their environment's pH as development progresses. This is accomplished by using a suitable buffer mechanism. In the microbiology lab, phosphate buffers are often employed; they allow medium to minimize pH shifts when acid or alkali are created.

Greater microbial growth will occur in places with more oxygen and in areas with essential nutrients than in areas with less oxygen. It may be challenging to regulate the amount of oxygen in a space, but depriving regions of food and other nutrients can starve off germs and keep a structure free of other pests as well. In addition to water, microbes often need certain gases in the air, which they consume to create necessary nutrition. Both oxygen and nitrogen are essential elements. Many microbes need high oxygen concentrations to exist, whereas others actually thrive in low oxygen environments. There is a large range in between these two extremes that may prefer more or less oxygen and may thrive equally well in either situation.

Most living forms rely on oxygen for existence and development, and it is a significant component of our atmosphere (20%). These creatures are known as aerobes. However, not all creatures are aerobes; certain anaerobes can exist without oxygen, and for some of them, this is absolutely necessary. By cultivating bacteria in thioglycolate tube cultures, we may quickly see how various organisms vary in their needs for molecular oxygen. An autoclaved thioglycolate medium with a little amount of agar is the foundation of a test-tube culture. This allows motile bacteria to move freely throughout the media. Strong reducing characteristics of thioglycolate cause the majority of the oxygen to be flushed out during autoclaving. The test-related bacterial cultures are injected into the tubes and then incubated at the proper temperature. From the top, oxygen progressively permeates the thioglycolate tube culture over time. In the region where the oxygen concentration is optimal for the development of that specific organism, bacterial density rises.

In environments with anaerobic conditions, such as deep soil deposits, calm waters, and the bottom of the deep ocean where there is no photosynthetic life, several obligate anaerobes may be discovered. Animals' digestive tracts naturally include anaerobic environments. A significant

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portion of the microorganisms in the human gut are obligatory anaerobes, mostly Bacteroidetes. When tissues are not supplied with blood circulation, transient anaerobic conditions develop; the tissues perish and serve as a perfect habitat for obligate anaerobes. The rod-shaped, gram-positive obligate anaerobe Clostridium spp. is another kind found in the human body. They can survive in the presence of oxygen thanks to their capacity to produce endospores. *C. difficile*, sometimes known as C. diff, is one of the main causes of infections acquired in a medical setting. Long-term use of antibiotics for other diseases raises the risk of subsequent *C. difficile* infection in a patient. Treatment with antibiotics upsets the delicate balance of gastrointestinal microbes, allowing *C. difficile* to colonise the gut and significantly inflaming the colon. Other clostridia that may cause harmful infections include *C. perfringens*, which can lead to gas gangrene, and *C. tetani*, the tetanus-causing bacterium. The infection begins in necrotic tissue in both situations (dead tissue that is not supplied with oxygen by blood circulation). Deep puncture wounds are linked to tetanus because of this. Gangrene is always a risk when there is no circulation and tissue death.

Equipment specific to obligatory anaerobe research is needed. Anaerobic bacteria must be cultured in anoxic conditions by law. Culture in an anaerobic jar is the most typical method. Chemical packs that remove oxygen from the air and release carbon dioxide are used in anaerobic jars (CO_2). An enclosed space where all oxygen has been eliminated is known as an anaerobic chamber. Handling the cultures without exposing them to air is made possible using gloves that are sealed to the box's holes. The top of tube A is where all the growth is visible. The bacteria are obligatory (strict) aerobes, meaning they need a lot of oxygen to develop. The reverse of tube A is how tube B appears. At the base of tube B, bacteria flourish. These are oxygen-sensitive obligate anaerobes. As is characteristic for facultative anaerobes, Tube C exhibits significant development at the tube's top and growth throughout the tube. If there is a suitable electron acceptor other than oxygen and the organism is capable of performing anaerobic respiration, then facultative anaerobes may develop even in the absence of oxygen by depending on fermentation or anaerobic respiration. The presence of oxygen had no effect on the aerotolerant anaerobes in tube D. They typically have a fermentative metabolism, thus they do not need oxygen, but they are not negatively affected by the presence of oxygen as obligate anaerobes are. A "Goldilocks" society is shown in Tube E on the right. For development to occur, the oxygen concentration must be precisely right—neither too high nor too low. These bacteria, known as microaerophiles, need a minimum of 1% to 10% oxygen for growth, which is far less than the 21% prevalent in the atmosphere.

Examples of obligate aerobes are Mycobacterium tuberculosis, the causative agent of tuberculosis and Micrococcus luteus, a gram-positive bacterium that colonizes the skin. Neisseria meningitidis, the causative agent of severe bacterial meningitis, and N. gonorrhoeae, the causative agent of sexually transmitted gonorrhea, are also obligate aerobes. Aerobic organisms require oxygen to act as a terminal electron acceptor in their respiratory chains. Such organisms, when grown in laboratory culture, must therefore be provided with enough oxygen to sat-isfy their requirements. For a shallow layer of medium such as that in a petri dish, sufficient oxygen is available dissolved in surface moisture. In a deeper culture such asa flask of broth however, aerobes will only grow in the surface layers unless additional oxygen is provided (oxygen is poorly soluble in water). This is usually done by shaking or mechanical stirring.

Bacteria have a minimum, optimum, and maximum temperature for growth and can be divided into 3 groups based on their optimum growth temperature: psychrophils, mesophils, thermophils, or hyperthermophils. Bacteria show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups: obligate aerobes, microaerophils,

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obligate anaerobes, aerotolerant anaerobes, or facultative anaerobes. Microorganisms can be placed in one of the following groups based on their optimum pH requirements: neutrophiles, acidophiles, or alkaliphiles. A bacterium's osmotic environment can affect bacterial growth. Bacteria can be grouped according to their energy source as phototrophs or chemotrophs. Bacteria can be grouped according to their carbon source as autotrophs or heterotrophs.Combining their nutritional patterns, all organisms in nature can be placed into one of four separate groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs. Bacteria also need a nitrogen source, various minerals, and water for growth. Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious.

Carbon dioxide

We saw that autotrophic organisms are able to use carbon dioxide as a carbon source; when grown in culture, these are provided with bicarbonate in their growth medium or incubated in a CO2-enriched atmosphere. However, heterotrophic bacteria also require small amounts of carbon dioxide, which is incorporated into var-ious metabolic intermediates. This dependency can be demonstrated by the failure of these organisms to grow if carbon dioxide is deliberately removed from the atmosphere.

Osmotic pressure

Osmosis is the diffusion of water across a semipermeable membrane from a less con-centrated solution to a more concentrated one, equalising concentrations. The pressure required to make this happen is called the osmotic pressure. If a cell were placed in ahypertonic solution (one whose solute concentration ishigher), osmosis would lead to a loss of water from the cell (plasmolysis). This is the basis of using high con-centrations of salt or other solutes in preserving foods against microbial attack. In the opposite situation, wa-ter would pass from a dilute (hypotonic) solution into the cell, causing it to swell and burst. The rigid cell walls of bacteria prevent them from bursting; this, together with their minute size, makes them less sensitive to variationsin osmotic pressure than other types of cell. They are generally able to tolerate NaCl con-centrations of between 0.5 and 3.0 per cent. Haloduric (‘salt-tolerant’) bacteria are able to tolerate concentrations ten times as high, but prefer lower concentrations, whereas halophilic(‘salt-loving’) forms are adapted to grow best in conditions of high salinitysuch as those that prevail in the Dead Sea in the Middle East. In order to do this without plasmolysis occurring, they must build up a higher internal solute concentration, which they do by actively concentrating potassium ions inside the cell.

Light

Phototrophic organisms require light in order to carry out photosynthesis. In the lab-oratory, care must be taken that light of the correct wavelength is used, and that the source used does not also act as a heat source. Fluorescent light produces little heat, but does not provide the wavelengths in excess of 750 nm needed by purple and green photosynthetic bacteria.

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CHAPTER 14

Microbial Nutrition

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Carbon, energy, and electrons are the three things that all bacteria need. For the purpose of defining organisms, certain phrases are connected to the origin of each of these elements. Let's initially concentrate on carbon. All living things are made of carbon, and the basic building block of all macromolecules, including proteins, carbohydrates, lipids, and nucleic acids, is carbon. On the one hand, organisms may employ prefabricated, reduced organic materials as a carbon source. These are heterotrophs, sometimes known as "other eaters." As an alternative, they may use carbon dioxide (CO2), which would be converted from its inorganic state into an organic molecule. These are autotrophs, sometimes known as "self-feeders."

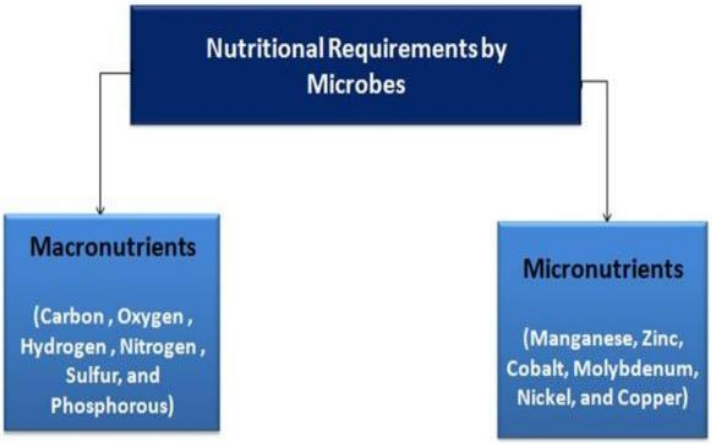

Figure 14.1: Nutritional Requirements by Microbes

There are two sources of energy that may be used: chemical energy or light energy. The sun is the source of light energy, while either organic or inorganic molecules may provide chemical energy. The term "light eater" refers to creatures that utilise light energy, whereas the term "chemical eater" refers to species that use chemical energy. Organic or inorganic materials may both provide chemical energy. An organism that consumes organic materials is known as an organotroph, whereas an organism that consumes inorganic materials is referred to as a lithotroph ("rock eater"). These concepts may all be combined to create a single phrase that describes how an organism satisfies its fundamental need for carbon, electrons, and energy.

Macronutrients

Cells also need a few other elements in adequate amounts in addition to carbon, hydrogen, and oxygen. In instance, nitrogen is necessary for the synthesis of proteins, nucleic acids, and a few

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other components in cells. Phosphorus is another element that cells need since it is an essential part of nucleic acids (think of the sugar-phosphate backbone!), phospholipids, and adenosine triphosphate, or ATP. A few vitamins and several amino acids both need sulphur, whereas potassium is required for enzymes and magnesium is employed to keep ribosomes and membrane stable. The macronutrients are all of these substances put together, together with C, H, and O.

Growing Points

If given a carbon supply and inorganic salts, certain microorganisms are capable of creating some organic compounds from scratch. Some organic chemicals must be present in the environment for other microorganisms to exist. The three types of these chemical compounds, purines and pyrimidines, vitamins, and amino acids, are together known as growth factors. Amino acids are the building blocks of protein (enzyme cofactors).

Nutritional Intake

A cell must penetrate its cell membrane to take in nutrients from the outside environment in order to sustain its functions. Numerous distinct transport systems are found in bacteria and archaea.

Non-Active Diffusion

Simple molecules and gases, such as CO2, O2, and H2O, are able to move across the cell membrane thanks to passive or simple diffusion. In this scenario, a gradient of concentration must exist, with a larger concentration of the drug outside the cell than within. Diffusion rates slow when more of the material is absorbed by the cell and the gradient of concentration flattens out.

Facilitate diffusion

In order to facilitate diffusion, carrier proteins are used instead of a concentration gradient, which places the substance's concentration outside the cell at a higher level (sometimes called permeases). These proteins, which are enmeshed in the cell membrane, create a hole or channel that allows bigger molecules to flow through the membrane barrier. The entry of molecules into the cell is halted if the concentration gradient disappears. Each carrier protein usually demonstrates selectivity, only allowing the transport of one kind of molecule or a group of related compounds.

Transportation Active

A cell's capacity to move materials against a concentration gradient is necessary for several forms of nutrition absorption (i.e. with a higher concentration inside the cell than outside). For the drug to be transported by carrier proteins anchored in the membrane, a cell must use metabolic energy. Active transportation is what this is. Carrier proteins are used in all forms of active transport. Chemical energy, such ATP, is used in primary active transport to power the movement. The ABC system is one instance of a system that makes use of ATP-Binding Cassette transporters. Each ABC transporter is made up of three distinct parts: Carrier proteins are membrane-spanning proteins that create a pore in the cell membrane. ATP binding regions hydrolyze ATP to provide the energy needed to cross the membrane. Substrate-binding proteins are peripheral proteins that bind to the proper substance to be transported and deliver it to the membrane-spanning proteins. The substrate-binding protein of gramme positive bacteria is connected to the cell membrane, while that of gramme negative bacteria is found in the periplasm of the cell.

Active secondary transportation

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Proton motive force energy is used in secondary active transport (PMF). When the cell moves electrons across energy-saving processes, a PMF, or ion gradient, forms. A proton gradient between the outside and the interior of the negatively charged cell is produced as positively charged protons gather along its exterior. Simple transport may occur in three distinct ways: uniport, symport, and antiport, and each method makes use of a different protein porter. Uniporters move a single chemical in one direction or the other across the membrane. Symporters move two things simultaneously through the membrane, usually a proton and a molecule. Similarly, but in the opposite manner, antiporters move two chemicals through the membrane. The other material is moved out of the cell when one enters.

Transferring a group

Using energy from an organic substance that is rich in energy but is not ATP, group translocation is a special sort of active transport. The drug being carried undergoes chemical modification during group translocation, which sets it apart from ABC transporters and simple transport. The phosphoenolpyruvate: sugar phosphotransferase system (PTS), which transports sugars into the cell using energy from the high-energy molecule phosphoenolpyruvate (PEP), is one of the most researched instances of group translocation. The transport procedure involves the transfer of a phosphate from the PEP to the incoming sugar.

Taking in iron

Iron is a micronutrient that limits development since microorganisms need it for the cytochromes and enzymes that they produce. However, owing to its insolubility, there isn't much free iron present in the environment. Siderophores are organic compounds that have developed in many bacteria and have a high affinity for binding or chelating ferric iron. The organism releases siderophores, which bind any ferric iron that is present in the environment. A particular receptor on the exterior of the cell subsequently binds the iron-siderophore complex, enabling the iron to enter the cell. Getting food and energy is the process of nutrition. Energy is necessary for all processes of development, reproduction, and upkeep. Bacteria primarily get their nutrition from carbon, nitrogen, water, phosphorus, iron, and a few inorganic salts. Bacteria kinds are listed below. The four main nutritional categories of bacteria are highlighted in the following sections. The varieties are: One, photoautotrophs Two. Photoheterotrophs Third, chemoautotrophs 4. Chemical heterotrophs

Principal Nutritional Bacteria

Photoautotrophs:

These microorganisms absorb solar energy and convert it to chemical energy. This process turns CO2 into carbs. Water serves as both the hydrogen donor and the producer of free oxygen. Chlorophyll is found in cells and its primary job is to absorb sunlight, like in the case of cyanobacteria. Free oxygen results from the process. However, some bacteria's photosynthesis uses a hydrogen donor other than water; as a result, oxygen is never created. Purple and green sulphur bacteria are both known to engage in this kind of oxygenic photosynthesis. These anaerobe photoautotrophic bacteria contain the pigments bacteriochlorophyll and bacteriovirdin. These pigments absorb light and break down carbon dioxide to create organic molecules during photosynthesis. Both carbon dioxide and hydrogen are obtained from sources other than water. Bacterioochlorophyll is a pigment found in the thylakoids of purple sulphur bacteria, which are

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positioned on the intracytoplasmic membrane. Sulfur compounds, such as Chromatiiun, provide energy to these bacteria. Theopedia rosea with thyospirillum

These bacteria, known as green sulphur bacteria, employed hydrogen sulphide (H2S) as a hydrogen donor. The reaction occurs in the presence of bacteriovirdin, bacteriopheophytin, or chlorobium chlorophyll, such as Chlorobium limicola, Chlorobacterium, etc. Inorganic hydrogen sources like sulphides and thiosulphates are used by these bacteria. As a result, these microorganisms are also called photolithographs. These bacteria are known as photoheterotrophs (Greek: photo = light, hetero = (an), other, and troph = sustenance). They are able to capture light energy but are unable to utilise carbon dioxide as their only carbon source. They get their needed carbon and electrons from organic substances found in the surrounding environment. They consume organic substances like carbohydrates, fatty acids, and alcohols as their main source of nutrition. The colour comes from bacteria, such as purple non-sulfur bacteria (Rhodospirillum, Rhodomicrobium, Rhodopseudomonas palustris).

Chemoautotrophs: These bacteria produce their own food without the need for light since they have the dark phase of photosynthesis (which lacks the light phase). With the aid of ambient oxygen, these bacteria oxidise certain inorganic materials. Exothermic energy is released during this reaction and is utilised to power the cell's synthesis operations. Carbon dioxide is the source of carbon. Chemosynthesis is the use of chemical energy to create organic food from inorganic materials in the absence of light. Inorganic nutrient recycling is greatly aided by the chemoautotrophic bacteria. These bacteria are often given names based on the composition of the substance they use as a source of energy, including

(a) Nitrifying Bacteria: These bacteria produce nitrate by oxidising ammonia, which gives them energy. Each of the two phases of the process is carried out by a unique group of bacteria. The bacteria Nitrosomonas and Nitrococcus oxidise ammonia in the first stage to produce nitrites. These nitrifying bacteria are widespread in soil and have a significant economic impact. Nitrification is the mechanism by which these two bacterial communities convert ammonia in the soil to nitrate. These bacteria utilise the energy produced by the nitrite and ammonia oxidations to carry out chemosynthesis (to make ATP by oxidative phosphorylation).

Sulphur bacteria (group b) produce energy either by oxidising H2S or elemental sulphur. Thiobacillus denitrificans, for example, uses the energy created during the oxidation of elemental sulphur to form sulphuric acid, which is then used by denitrifying sulphur bacteria. These bacteria live in fluids with inorganic iron compounds and convert ferrous compounds to ferric forms, such as Thiobacillus ferroxidans, Ferro bacillus, and Leptothrix.

Chemoheterotrophs: These microorganisms rely on organic substances including proteins, lipids, and carbohydrates for both carbon and energy. For these bacteria, both the source of carbon and the source of energy are largely the same. The vast majority of bacteria are chemo heterotrophs.

(i) Parasitic: These bacteria feed on the live hosts on whom they proliferate. Pathogens, such as Clostridium and Mycobacterium, are parasites that cause illness.

(ii) Saprophytic: These bacteria feed on dead things including fruits, vegetables, leaves, meat, faeces, corpses, and other non-living things. Protein breakdown occurs anaerobically and is known as putrefaction, whereas the breakdown of carbohydrates is known as fermentation. Putrefying bacteria include Bacillus mycoides, B. ramosus, and others.

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(iii) Symbiotic: These bacteria coexist closely with the organs of other species (higher plants and animals) in a manner that is beneficial to both the organisms involved. For instance, Rhizobium leguminosarum in the root nodules of the leguminous plants engages in this kind of symbiosis. This bacterium converts free atmospheric nitrogen into nitrogenous chemicals that plants may use. In exchange, the plant gives the bacteria nourishment and defence. Because cellulose is broken down by microorganisms in the stomachs of cows and goats, these animals can eat grass. Escherichia coli and other benign bacteria may be found in our own intestines.

Carbon and energy source categories

The source of carbon that an organism uses for metabolism and its energy source may be used to identify it. Auto- ("self") and hetero- ("other") are prefixes that allude to the origins of the carbon sources that different organisms might employ. Autotrophs are organisms that transform inorganic carbon dioxide (CO2) into organic carbon molecules. Autotrophs are organisms that are known to exist in plants and cyanobacteria. Contrarily, the more complex organic carbon molecules that autotrophs first provide to heterotrophs serve as their source of nutrition. Heterotrophic organisms include a wide variety of species, including the well-researched Escherichia coli prokaryote and humans.

Another way to recognise an organism is by the energy source it uses. While the transmission of electrons is the source of all energy, different organismal kinds have different sources of electrons. The terms "light" and "chemical" in the prefixes "photo-" and "chemo-" denote the different sources of energy used by organisms. Chemotrophs break chemical bonds to gain energy for electron transfer, while phototrophs derive their energy for electron transfer from light. Organotrophs and lithotrophs are the two categories of chemotrophs. Humans, fungus, and many prokaryotes are all organotrophs, which are chemotrophs that get their energy from organic chemicals. Chemotrophs known as lithotrophs—from the Greek litho, which means "rock"—get their energy from inorganic substances like hydrogen sulphide (H2S) and reduced iron. Lithotrophy is exclusive to microbes. The methods for obtaining carbon and energy may be combined to classify organisms based on their nutritional needs. Because they utilise organic molecules as both their carbon and electron sources, the majority of organisms are chemoheterotrophs.

Metabolic Oxidation and Reduction

Because the majority of the energy held in atoms and utilised to power fuel cell operations comes in the form of high-energy electrons, the transport of electrons between molecules is crucial. The cell can transmit and utilise energy progressively, that is, in little doses as opposed to in a single, devastating burst, thanks to the transmission of energy in the form of electrons. Oxidation processes involve the removal of electrons from donor molecules, leaving them oxidised; reduction reactions involve the addition of electrons to acceptor molecules, leaving them reduced. Oxidation and reduction can occur simultaneously because electrons may flow from one molecule to another. Redox reactions, often known as oxidation-reduction pairs of reactions, are these processes.

Energy Transporters: ATP, FAD, NAD+, and NADP+

Either by reducing electron carriers or in the bonds of adenosine triphosphate, the energy generated when the chemical bonds in nutrients break down may be retained (ATP). A select group of substances act as molecules that bind to and transport high-energy electrons through routes

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between other substances in biological systems. The main electron carriers that we shall take into consideration are nicotinamide adenine dinucleotide, nicotine adenine dinucleotide phosphate, and flavin adenine dinucleotide, all of which are derivatives of nucleotides. These substances are readily oxidised or reduced. The most prevalent mobile electron carrier employed in catabolism is nicotinamide adenine dinucleotide (NAD+/NADH). The molecule exists in two states: oxidised (NAD+) and reduced (NADH). Another significant electron carrier is nicotine adenine dinucleotide phosphate (NADP+), which is the oxidised version of a variation of NAD+ with an additional phosphate group. When reduced, NADP+ transforms into NADPH. Flavin adenine dinucleotide has two states: oxidised (FAD) and reduced (FADH2). In chemoheterotrophs, NAD+/NADH, FAD/FADH2, and NADP+/NADPH all play significant roles in the energy extraction from sugars during catabolism, whereas NADP+/NADPH is crucial for anabolic processes and photosynthesis. FADH2, NADH, and NADPH are often referred to as having reducing power together because of their capacity to provide electrons to a variety of chemical processes.

In order to securely store energy and release it only when necessary, a living cell must be able to manage the energy generated during catabolism. Adenosine triphosphate is used by living cells to do this (ATP). The flexible substance ATP is sometimes referred to as the "energy currency" of the cell since it may be utilised to meet any of the cell's energy requirements. Adenosine monophosphate (AMP), the fundamental unit of ATP, is made up of an adenine molecule linked to a ribose molecule and a single phosphate group. One of the nucleotides in RNA is AMP, and ribose, a five-carbon sugar, is also present in RNA. Adenosine diphosphate (ADP) is created by adding a second phosphate group to this core molecule, while ATP is created by adding a third phosphate group (Figure 8.3). It takes energy to phosphorylate a molecule, which is the addition of a phosphate group. Since phosphate groups are negatively charged and are organised in series in ADP and ATP, they repel one another. The ADP and ATP molecules are intrinsically unstable due to this attraction. So-called high-energy phosphate bonds are the bonds between the phosphate groups (one in ADP and two in ATP). Energy is released to power endergonic reactions when these high-energy bonds are broken to liberate one phosphate (known as inorganic phosphate [Pi]) or two linked phosphate groups (known as pyrophosphate [PPi]) from ATP via a process known as dephosphorylation.

Structure and Activity of Enzymes

A catalyst is a material that aids in accelerating a chemical process. Catalysts are reusable since they are not consumed or altered during chemical processes. While proteins known as enzymes operate as catalysts for biological events within cells, inorganic substances may act as catalysts for a variety of chemical reactions. Thus, enzymes are crucial in regulating cellular metabolism. An enzyme works by reducing a chemical reaction's internal activation energy. The energy required to build or break chemical bonds and change reactants into products is known as activation energy (Figure 8.5). By attaching to the reactant molecules and retaining them so that the reaction proceeds more quickly, enzymes reduce the activation energy. The substances that an enzyme binds to are referred to as substrates, and the area of the enzyme where the substrate binds is referred to as the enzyme's active site. A highly specialised chemical environment is created inside the active site due to the properties of the amino acids nearby, which makes it possible to bind, although temporarily, to a particular substrate (or substrates). Enzymes are renowned for their specificity as a result of this jigsaw puzzle-like fit between an enzyme and its substrates. In reality, much as a rubber glove conforms to a hand placed into it, an enzyme's structure alters somewhat

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when it binds to its substrate(s) to find the optimal fit between the transition state (a structural intermediate between the substrate and product) and the active site. Induced fit refers to the simultaneous creation of the transition state and the active-site alteration in the presence of the substrate (Figure 8.6). Overall, each substrate and hence each chemical reaction has an enzyme that is exactly linked to it; yet, there is also considerable flexibility. Some enzymes can interact with a variety of substrates that have structural similarities.

Local environmental factors, such as pH, substrate concentration, and temperature, may have an impact on an enzyme's activity. Although raising or lowering the temperature outside of an ideal range might disrupt chemical bonds inside the active site and make them less well-suited to bind substrates, doing so typically improves reaction speeds, whether enzyme-catalyzed or not. Like all biological molecules, enzymes will ultimately denature at high temperatures, losing their three-dimensional structure and functionality. Enzymes are likewise ideally adapted to operate within a certain pH range, and much as with temperature, very acidic or basic ambient pH values may lead to the denature of enzymes. Because the amino-acid side chains at the active site have their own acidic or basic characteristics that are ideal for catalysis, they are sensitive to pH changes. Substrate concentration is another element that affects enzyme activity; enzyme activity rises with increasing substrate concentrations until it reaches a saturation point when it can no longer bind more substrate. Overall, enzymes are designed to function optimally in the environments where their producing species inhabit. For instance, human infections have enzymes that function best at 37°C, but bacteria that live in hot springs prefer high temperatures. Similar to this, whereas most organisms develop enzymes that function best at a neutral pH, microorganisms that thrive in acidic environments create enzymes that are optimised for low pH conditions, enabling them to proliferate there.

Many enzymes need particular nonprotein helper molecules to which they are attached, either permanently via stronger covalent connections or momentarily through ionic or hydrogen interactions, in order to function properly or even at all. For each of these enzymes, binding to these compounds encourages optimum conformation and performance. Cofactors and coenzymes are two examples of assistance molecules. Cofactors are inorganic ions that assist maintain the conformation and activity of enzymes. Examples include iron (Fe^{2+}) and magnesium (Mg^{2+}). The enzyme that creates DNA molecules, DNA polymerase, is one example of an enzyme that needs a metal ion as a cofactor in order to operate. DNA polymerase requires a bonded zinc ion (Zn^{2+}) in order to function. Coenzymes are organic support molecules necessary for the activity of enzymes. They are not eaten, making them reusable like enzymes. Dietary vitamins are the most typical sources of coenzymes. Some vitamins function as coenzyme precursors, while others function as coenzymes themselves. Coenzyme A (CoA), for example, frequently binds to the active site of an enzyme, assisting in the chemistry of the change from a substrate to a product (Figure 8.7). In such circumstances, an enzyme is referred to as an apoenzyme and is inactive if it is missing a crucial cofactor or coenzyme. On the other hand, an enzyme is said to be a holoenzyme and is active if it has the required associated cofactor or coenzyme. Common coenzymes that supply high-energy electrons or phosphate groups, respectively, that bind to enzymes and activate them include NADH and ATP.

Enzyme Inhibitors

It is possible to control enzymes in ways that increase or decrease their activity. Different sorts of chemicals may increase or hinder enzyme activity, and there are several strategies for doing so. A

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competitive inhibitor is a chemical that is sufficiently similar to a substrate to compete with it for binding to the active site by obstructing the substrate's ability to attach. The inhibitor concentration must be close to the substrate concentration in order for a competitive inhibitor to work. A nice illustration of competitive rivalry is seen in sulfa medications. Because they bind to the active site of an enzyme along the bacterial folic acid production pathway, they are utilised to treat bacterial infections. A sulfa medication blocks the production of folic acid when it is present in high enough concentrations. As a result, bacteria are unable to proliferate because they cannot produce DNA, RNA, or proteins. Because we get our folic acid from food, humans are unaffected.

A noncompetitive (allosteric) inhibitor, on the other hand, binds to the enzyme at an allosteric site, which is different from the active site, yet nonetheless manages to prevent substrate binding to the active site by causing a conformational shift that lowers the enzyme's affinity for its substrate. The concentration of inhibitors required for noncompetitive inhibition is often significantly lower than the substrate concentration since only one inhibitor molecule is required per enzyme for effective inhibition. As an alternative to allosteric inhibitors, allosteric activators bind to regions of an enzyme other than the active site, causing a conformational shift that enhances the enzyme's active site(s) for its substrate's affinity (s).

An essential strategy for controlling metabolic pathways involved in both catabolism and anabolism is allostery control. Cells have evolved to exploit the byproducts of their own metabolic activities for feedback inhibition of enzyme activity in a very effective and elegant manner. A route product is used to control the synthesis of other pathway products via feedback inhibition. The cell slows down synthesis during anabolic or catabolic responses in response to the overabundance of certain products. There are several enzyme mechanisms that can degrade carbohydrates in order to release energy from ATP bonds. A lot of catabolic pathways also result in the production of intermediate molecules that serve as the basis for anabolism. It's crucial to comprehend these processes for a number of reasons. First off, studying metabolism in simpler to control bacteria like E. coli may teach us a lot about human metabolism since the key metabolic pathways are shared by a variety of chemoheterotrophic animals. Second, because both human and animal pathogens are chemoheterotrophs, understanding the specifics of these bacteria's metabolism, including potential variations between bacterial and human pathways, is important for both the diagnosis of pathogens and the development of antimicrobial treatments that specifically target particular pathogens. The precise processes involved in chemoheterotrophic metabolism may also be studied in order to compare them to other, more novel metabolic techniques used by bacteria. Both chemoheterorophs and chemoautotrophs employ many of the same mechanisms, despite the fact that the chemical source of the electrons that initiate electron transfer differs between the two kinds of organisms.

The standard illustration used to explain metabolic topics to students is carbohydrate catabolism. Our examples of metabolism for chemoheterotrophs begin with the breakdown of polysaccharides like glycogen, starch, or cellulose. The hydrolysis of the glycosidic bonds between the glucose monomers in these polymers by enzymes like amylase, which breaks down starch or glycogen, and cellulases, which breaks down cellulose, releases glucose for further catabolism.

Glycolysis

Glycolysis is the most prevalent mechanism for the catabolism of glucose in bacteria, eukaryotes, and the majority of archaea; it generates energy, reduced electron carriers, and precursor molecules for cellular metabolism. Glycolysis is a process that occurs in some form in every living thing,

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which suggests that this mechanism is an old, universal metabolic activity. Although glycolysis on its own does not require oxygen, it can be combined with other aerobic or anaerobic metabolic processes. The cytoplasm of prokaryotic and eukaryotic cells is where glycolysis occurs. It starts with one glucose molecule, which has six carbons, and concludes with two pyruvate molecules, which have three carbons. After glycolysis, pyruvate may be further broken down to release additional energy by aerobic or anaerobic respiration, but for many species, including many bacteria, respiration may not be an option; for these organisms, glycolysis may be the only way to produce ATP.

The Embden-Meyerhof-Parnas (EMP) route, so called in honour of Gustav Embden (1874-1933), Otto Meyerhof (1884-1951), and Jakub Parnas, is the kind of glycolysis most often seen in microorganisms and present in vertebrates (1884–1949). The EMP route for glycolysis involves two separate stages (Figure 8.10). The glucose molecule is modified in the first stage of the route, known as the energy investment phase, using energy from two ATP molecules so that the six-carbon sugar molecule may be equally divided into two phosphorylated three-carbon molecules known as glyceraldehyde 3-phosphate (G3P). The energy payout phase, which is the second step of the route, harvests energy by oxidising G3P to pyruvate, creating four ATP molecules, and reducing two molecules of NAD+ to two molecules of NADH utilising glucose-derived electrons.

One of the two processes for making ATP, substrate-level phosphorylation, is responsible for creating the ATP molecules generated during the energy payout stage of glycolysis. When an organic molecule's phosphate group is removed during substrate-level phosphorylation, it is immediately transferred to an accessible ADP molecule, creating ATP. The intermediate molecules' high-energy phosphate groups are combined with ADP during glycolysis to produce ATP.

Additional Glycolytic Routes

Unless otherwise stated, we refer to the EMP route, which is utilised by both mammals and many microbes, when we talk about glycolysis. Some prokaryotes, nevertheless, use different glycolytic processes. The Entner-Doudoroff (ED) route, so called for its discoverers Nathan Entner and Michael Doudoroff, is a significant alternative (1911–1975). Some bacteria, such as the opportunistic gram-negative pathogen Pseudomonas aeruginosa, exclusively have the ED route for glycolysis, but E. coli may utilise either the ED pathway or the EMP pathway. The pentose phosphate route (PPP), also known as the phosphogluconate pathway or the hexose monophosphate shunt, is a third kind of glycolytic process that takes place in all cells and is significantly distinct from the first two pathways. The PPP may be the oldest global glycolytic route, according to the evidence. Nucleotides and amino acids are biosynthesized using the PPP's intermediates. As a result, this glycolytic process can be preferred when the organism needs to synthesise proteins or nucleic acids, respectively.

The Krebs cycle and glycolysis are the two glucose catabolism processes that produce ATP via substrate-level phosphorylation. However, oxidative phosphorylation, a different process that takes place during cellular respiration, is where the majority of ATP is produced. When electrons are moved from NADH and FADH2, which are produced in the Krebs cycle, transition reaction, and glycolysis, to a final inorganic electron acceptor via a sequence of chemical processes, cellular respiration occurs (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration). Prokaryotic cells' inner cell membranes or eukaryotic cells' mitochondrial inner membranes include specific protein complexes where these electron exchanges take occur.

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By creating an electrochemical gradient across the membrane with the energy of the electrons, oxidative phosphorylation may produce ATP.

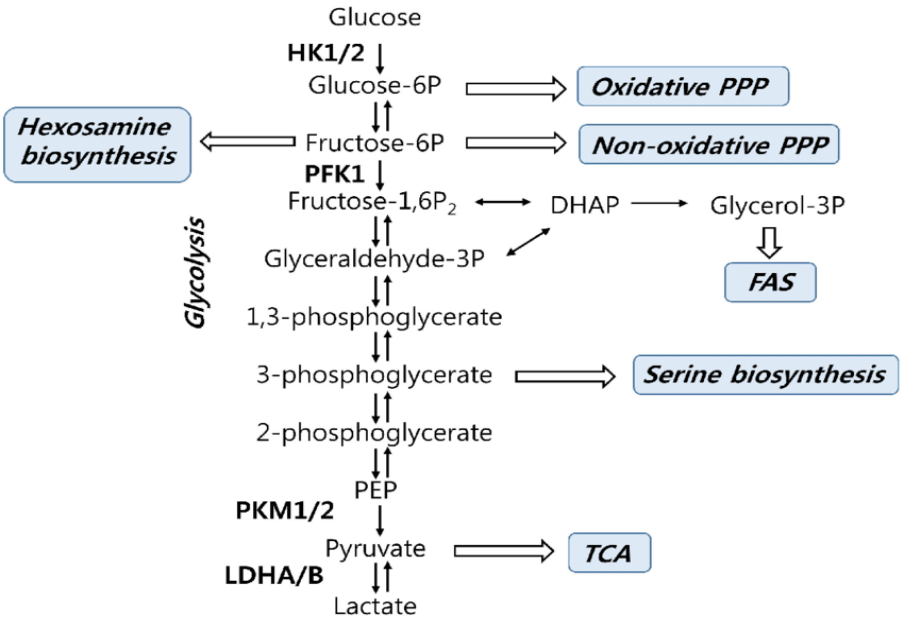

Figure 14.2: Additional Glycolytic Routes

Electron Transport System

The electron transport system (ETS) is the last component involved in the process of cellular respiration; it comprises a series of membrane-associated protein complexes and associated mobile accessory electron carriers (Figure 8.15). Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons from NADH and FADH2 are passed rapidly from one ETS electron carrier to the next. These carriers can pass electrons along in the ETS because of their redox potential. For a protein or chemical to accept electrons, it must have a more positive redox potential than the electron donor. Therefore, electrons move from electron carriers with more negative redox potential to those with more positive redox potential. The four major classes of electron carriers involved in both eukaryotic and prokaryotic electron transport systems are the cytochromes, flavoproteins, iron-sulfur proteins, and the quinones.

The oxygen molecule (O_2) that the last ETS carrier reduces to water (H2O) at the conclusion of the ETS is the final electron acceptor (i.e., the one bearing the maximum positive redox potential) in aerobic respiration. To help with diagnosis, cytochrome oxidase, an electron transporter, may distinguish between several species of bacteria. For instance, the oxidase test can identify the gram-negative opportunist Pseudomonas aeruginosa and the gram-negative cholera-causing Vibrio cholerae as using cytochrome c oxidase, while other gram-negative Enterobacteriaceae, like E. coli, are negative for this test because they produce different types of cytochrome oxidase.

A number of conditions, including any one or more of the following, prevent aerobic respiration from occurring. For converting electrons to oxygen at the conclusion of the electron transport system, the cell is deficient in the genes that would produce the necessary cytochrome oxidase. The cell is deficient in genes that would make enzymes that would lessen the very harmful consequences of hazardous oxygen radicals generated during aerobic respiration, including

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hydrogen peroxide (H_2O_2) or superoxide (O^{2-}). Aerobic respiration cannot take place in the cell because there is not enough oxygen present. Anaerobic respiration, which uses an inorganic molecule other than oxygen as a final electron acceptor, is one potential substitute for aerobic respiration. Bacteria and archaea use a variety of anaerobic respiration techniques. Important soil bacteria called denitrifiers employ nitrate (NO^{3-}) and nitrite (NO^{2-}) as final electron acceptors to produce nitrogen gas (N_2). Numerous aerobically respiring bacteria, including *E. coli*, convert to utilising nitrate as a final electron acceptor and generating nitrite when oxygen levels are low.

The Krebs cycle is often intact in microbes that use anaerobic respiration, allowing them to access the energy contained in the NADH and FADH2 molecules produced. A different set of ETS carriers, comprising unique complexes for electron transfer to their ultimate electron acceptors, are used by anaerobic respirers, as encoded by their genomes. These electron transport mechanisms provide smaller electrochemical gradients, which results in less ATP being produced during anaerobic respiration.

Although an electron loses energy with each transfer across the ETS, certain transfers allow the electron to preserve this energy by utilising it to push hydrogen ions (H+) across a membrane. H+ is pumped from the mitochondrial matrix across the inner mitochondrial membrane and into the intermembrane space in prokaryotic cells (referred to as the periplasmic space in gram-negative and gram-positive bacteria). In eukaryotic cells, H+ is pumped from the mitochondrial matrix across the outer mitochondrial membrane and into the intermembrane space. Being negatively charged (electrically) and having a larger concentration (chemically) on one side of the membrane, H+ ions are unevenly distributed across the membrane, creating an electrochemical gradient. The proton motive force is the name given to an electrochemical gradient created by the concentration of H+, or a proton, on one side of the membrane in comparison to the other (PMF). The H+ ions involved cause a pH gradient to be created, with the side of the membrane with the larger concentration of H+ being more acidic. The PMF may be utilised to drive a number of energetically unfavourable activities, including as the transfer of nutrients and the rotation of flagella for motility, in addition to its usage to produce ATP, as explained in this chapter.

The electrochemical gradient's potential energy, produced by the ETS, causes the H+ to spread across a membrane (the plasma membrane in prokaryotic cells and the inner membrane in mitochondria in eukaryotic cells). ATP synthase, a membrane-bound enzyme complex, is required for the movement of hydrogen ions across the membrane, which is known as chemiosmosis (Figure 8.15). Similar to water that builds up on one side of a dam and flows through it when the dam is opened, this movement has a propensity to happen. A complex protein called ATP synthase functions as a tiny generator, turning by the force of the H+ diffusing through the enzyme, down their electrochemical gradient from where there are many mutually repelling H+ to where there are fewer H+. This process is similar to how a hydroelectric dam's intake and generator work together. H+ moves from the intermembrane gap to the mitochondrial matrix in eukaryotic mitochondria but from the outside of the cytoplasmic membrane into the cytoplasm of prokaryotic cells. By oxidative phosphorylation, a second method of producing ATP that taps into the potential energy contained inside an electrochemical gradient, the moving pieces of this molecular machine create ATP from ADP and inorganic phosphate (Pi).

When glucose is broken down, different amounts of ATP are produced. For instance, the capacity of the electron transport system complexes to pump hydrogen ions across the membrane differs between various species of animals. In aerobic respiration in mitochondria, the passage of electrons

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from one molecule of NADH creates enough proton motive force to produce three ATP molecules by oxidative phosphorylation, but the transit of electrons from one molecule of FADH2 only produces two ATP molecules. Thus, during glycolysis, the transition reaction, and the Krebs cycle, 10 NADH molecules are produced for every glucose molecule, and these molecules contain enough energy to produce 30 ATP molecules, as opposed to two FADH2 molecules, which are produced for every glucose molecule, and which carry out the same functions but only produce four ATP molecules. With four molecules produced by substrate-level phosphorylation and 34 molecules produced by oxidative phosphorylation, the total theoretical maximum yield of ATP produced during the full aerobic respiration of glucose is 38 molecules (Figure 8.16). Realistically, the total ATP yield is typically lower, ranging from one to 34 ATP molecules, depending on whether the cell uses aerobic or anaerobic respiration. Additionally, in eukaryotic cells, some energy is required to transport intermediates from the cytoplasm into the mitochondria, which reduces ATP yield.

Due to one or more of the following factors, many cells cannot carry out respiration. Any suitable, inorganic final electron acceptor that is required for cellular respiration is not present in adequate quantities in the cell. The electron transport system's complexes and electron carriers cannot be produced by the cell because it lacks the necessary genes. No genes are present in the cell to produce any of the Krebs cycle enzymes.

The other two criteria are genetically fixed, while the absence of a suitable inorganic final electron acceptor is environment-dependent. As a result, many prokaryotes are permanently incapable of respiration, even in the presence of oxygen, including individuals from the clinically significant genus Streptococcus. On the other hand, many prokaryotes are facultative, which means that, should the environmental conditions change to provide an appropriate inorganic final electron acceptor for respiration, organisms containing all the necessary genes will switch to cellular respiration for glucose metabolism since respiration allows for much higher ATP production per glucose molecule.

In the absence of respiration, glycolysis—the cell's only method of generating any ATP—must proceed in order for NADH to be reoxidized to NAD+ and reused as an electron carrier. In certain biological systems, a procedure known as fermentation is used to utilise an organic molecule (often pyruvate) as the ultimate electron acceptor. Beyond the ATP generated during glycolysis by substrate-level phosphorylation, no extra ATP is directly created during fermentation because it lacks an electron transport mechanism. The maximum number of ATP molecules per glucose produced by fermenting organisms, often known as fermenters, is two. The ultimate electron acceptors and strategies for ATP generation in aerobic respiration, anaerobic respiration, and fermentation are contrasted in Table 8.2. Please take note that the Embden-Meyerhof-Parnas route is assumed for the quantity of ATP molecules reported for glycolysis. Indicated is the ratio of ATP molecules produced by substrate-level phosphorylation (SLP) to oxidative phosphorylation (OP).

The term "lactic acid bacteria" (LAB) refers to a group of gram-positive bacteria that includes strains of the genera *Lactobacillus*, *Leuconostoc*, and *Streptococcus*. The very acidic environment created by lactic acid fermentation during the making of yoghurt and cheese denatures the milk's proteins, resulting in its solidification. When lactic acid is the sole result of the fermentation process, it is referred to as homolactic fermentation. This is the situation when the bacteria *Lactobacillus delbrueckii* and *S. thermophiles* are employed to make yoghurt. However, because to their usage of the branching pentose phosphate route for glycolysis rather than the EMP

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pathway, many bacteria engage in heterolactic fermentation, yielding a combination of lactic acid, ethanol, and/or acetic acid, as well as CO_2. *Leuconostoc mesenteroides* is a crucial heterolactic fermenter that is utilised to sour plants like cucumbers and cabbage to make pickles and sauerkraut, respectively.

Additionally crucial to medicine are lactic acid bacteria. The body creates settings with low pH levels, which prevent germs from colonising and expanding there. For instance, the vaginal microbiome is mostly made up of lactic acid bacteria; however, when these bacteria are diminished, yeast may grow and result in a yeast infection. Probiotics' key ingredient is lactic acid bacteria, which are also crucial for maintaining the wellbeing of the gastrointestinal system. The fermentation of alcohol, which results in ethanol, is another well-known fermentation process. In the first process, the pyruvate decarboxylase enzyme removes a carboxyl group from pyruvate, releasing CO_2 gas and creating the two-carbon acetaldehyde molecule. By transferring an electron from NADH to acetaldehyde in the second reaction, which is mediated by the enzyme alcohol dehydrogenase, ethanol and NAD+ are produced. Alcoholic drinks are made by using the yeast Saccharomyces cerevisiae to ferment pyruvate into ethanol, and CO_2 is also produced during this process, which causes bread products to rise. Production of biofuels outside the food business depends on the fermentation of plant-based ethanol.

Prokaryotes engage in a variety of additional fermentation processes in addition to lactic acid and alcohol fermentation in order to guarantee a sufficient supply of NAD+ for glycolysis (Table 8.3). These pathways are necessary for glycolysis to take place and for the production of ATP from the breakdown of glucose. It should be noted that most types of fermentation, with the exception of homolactic fermentation, result in the production of gas, most often CO_2 and/or hydrogen gas. The synthesis of various organic acids occurs along each of these many fermentation channels, which all contribute to the distinctive taste of a specific fermented food product. Many of these diverse kinds of fermentation pathways are also used in the food industry. For instance, Swiss cheese's characteristic taste is a result of the propionic acid created during the propionic acid fermentation process. Outside of the food business, a number of fermentation products are significant economically. For instance, during the fermentation of acetone, butanol, and ethanol, chemical solvents like acetone and butanol are created. Mixed acid fermentation is utilised to create the complex organic medicinal molecules used in antibiotics (like penicillin), vaccinations, and vitamins. In the lab, fermentation products are used to distinguish between diverse bacteria for diagnostic reasons. Enteric bacteria, for instance, are recognised for their capacity to carry out mixed acid fermentation, decreasing pH, which may be identified with a pH indicator. Similarly, it is possible to identify the bacterial synthesis of acetoin during the fermentation of butanediol. An inverted Durham tube that collects generated gas in a broth culture also demonstrates how gas is formed during fermentation.

According to the substrates they can ferment, microbes may also be classified. In contrast to some of its near gram-negative cousins, E. coli can digest lactose and produce gas. The pathogenic enterohemorrhagic O157:H7 strain of E. coli is distinguished from other E. coli strains by its inability to ferment the sugar alcohol sorbitol, which is employed as a marker for its presence. Last but not least, Staphylococcus aureus that ferments mannitol differs from staphylococci that do not.

Fat Catabolism

In animals, triglycerides are a kind of long-term energy storage. Glycerol and three fatty acids are the ingredients. All organisms, with the exception of archaea, have phospholipids as part of their

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cell and organelle membranes. Triglycerides and phospholipids have structures that are identical, with the exception that one of the fatty acids is substituted by a phosphorylated head group. Triglycerides and phospholipids are initially broken down by releasing fatty acid chains from the three-carbon glycerol backbone (and/or the phosphorylated head group in the case of phospholipids). Lipases and phospholipases, respectively, catalyse the events that break down triglycerides and involve phospholipids. These enzymes help make certain bacteria more virulent, including the bacterium Staphylococcus aureus and the fungal species Cryptococcus neoformans. These bacteria employ phospholipases to break down the lipids and phospholipids in host cells, and then they utilise the catabolic byproducts as a source of energy (see Virulence Factors of Bacterial and Viral Pathogens).

Glycerol and fatty acids, which are the end results of lipid catabolism, may be broken down even further. It is simple to convert glycerol to glycerol-3-phosphate, which may then be further transformed to glyceraldehyde 3-phosphate by glycolysis. The released fatty acids undergo catabolism in a procedure known as -oxidation, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains, reducing NAD+ and FAD to produce NADH and $FADH_2$, respectively, whose electrons can be used to make ATP through oxidative phosphorylation. Coenzyme A transports the acetyl groups generated during -oxidation to the Krebs cycle, where they are degraded to CO_2 via substrate-level phosphorylation and yield ATP as well as extra NADH and $FADH_2$ molecules.

Certain bacteria have the ability to break down additional lipid types. For instance, the capacity of certain bacteria to breakdown cholesterol, such as Mycobacterium TB, adds to their pathogenicity. Enzymatic degradation of cholesterol's side chains can be done with relative ease, but the degradation of the remaining fused rings presents greater difficulties. Specific enzymes help in a multi-step process where the four fused rings are successively split. The end products, including pyruvate, may then be further catabolized in the Krebs cycle.

Humans and other heterotrophic organisms, such as E. coli, depend on the chemical energy that is mostly present in carbohydrate molecules. As phototrophic organisms transform solar energy (sunlight) into chemical energy via the biochemical process of photosynthesis, a large portion of these carbohydrates are created. Despite the fact that photosynthesis is often associated with plants, microbial photosynthesis is also an important source of chemical energy, supporting a variety of varied ecosystems. Microbial photosynthesis will be the main topic of this section. The two consecutive phases of photosynthesis are the light-dependent processes and the light-independent reactions. In light-dependent processes, solar energy is transformed into stored chemical energy by pigment molecules in photosynthetic membranes. Although CO_2 is used to drive the assembly of sugar molecules in the light-independent reactions, the chemical energy generated by the light-dependent reactions is still used, making the reactions still light-dependent because the short-lived products of the light-dependent reactions are required to drive them. To store energy temporarily, the light-dependent processes result in ATP and either NADPH or NADH. In order to drive the energetically unfavourable process of "fixing" inorganic CO_2 in an organic form, sugar, these energy carriers are utilised in the light-independent processes.

Ecosystems experience a directed flow of energy, which enters as inorganic compounds for chemoautotrophs or as sunlight for phototrophs. The six most frequent elements found in organic molecules are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulphur. They may persist for extended periods of time in the atmosphere, on land, in water, or below the surface of the planet

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in a variety of chemical forms. The earth's cycle of elements is influenced by geologic processes such erosion, river drainage, continental plate movement, and weathering. A biogeochemical cycle is the term used to describe the recycling of inorganic materials between living organisms and their nonliving environment. Geology and chemistry play significant roles in the study of this process. Here, we shall concentrate on the involvement of microbes in these cycles, which play roles at each phase and commonly combine reduced and oxidised forms of molecules.

Carbon Cycle

Given its abundance and inclusion in all organic compounds, carbon is one of the elements that is most crucial to the existence of living things. The relationship between species in diverse ecosystems is best shown by the carbon cycle. In order to generate multicarbon, high-energy organic compounds like glucose, autotrophs utilise atmospheric CO_2, a fully oxidised form of carbon, as their fundamental building block. This movement of carbon between heterotrophs and autotrophs occurs both inside and across ecosystems. In order to covalently bond carbon atoms together into reduced organic compounds, photoautotrophs and chemoautotrophs, respectively, use energy from the sun and from inorganic chemical compounds. These organic compounds' energy can then be accessed through the processes of respiration and fermentation (Figure 8.24).

Overall, the heterotrophs (which generate CO_2 as a consequence of respiration or fermentation) and the autotrophs constantly trade CO_2 (which use the CO_2 for fixation). In addition to respiring or fermenting, autotrophs also utilise the organic molecules they produce for their own metabolic requirements rather than fixing carbon for heterotrophs. Methanotrophs are bacteria and archaea that utilise methane as a source of carbon. When CO_2 is used by archaea referred to as methanogens as a terminal electron acceptor in anaerobic respiration, reduced one-carbon molecules like methane accumulate in certain anaerobic conditions. Some methanogens also convert the two carbons in acetate into methane and carbon dioxide (CO_2). Methanogenesis causes methane to accumulate in both natural anaerobic soil and aquatic habitats. Methanogenesis also results from animal husbandry since methanogens are part of the typical ruminant microbiome. Because methane is a potent greenhouse gas and methanotrophs contribute to the reduction of atmospheric methane levels, environmental methane buildup resulting from methanogenesis is important.

Carbon Cycle

Nitrogen is a component of several biological macromolecules, such as proteins and nucleic acids, yet it is challenging to enter living things. Prokaryotes are crucial players in the nitrogen cycle (Figure 8.25), converting nitrogen into different forms for their own purposes and indirectly assisting other species. Even though this molecule makes up around 78% of the atmosphere, plants and phytoplankton cannot take up nitrogen from it (where it resides as firmly linked, triple covalent N2). Free-living and symbiotic bacteria are the primary source of nitrogen in the biosphere. Through nitrogen fixation, these bacteria add nitrogen to their macromolecules. In aquatic habitats, cyanobacteria convert nitrogen gas from inorganic sources into ammonia (NH3), which is readily absorbed into biological macromolecules. Rhizobium bacteria (Figure 8.1) also fix nitrogen and coexist symbiotically with legumes (such as beans, peanuts, and peas) in the root nodules, giving them the organic nitrogen, they need in return for the fixed carbon they get as sugar. Nitrogen may also be fixed by free-living bacteria, such as those in the genus Azotobacter.

Microbes use the three processes of ammonification, nitrification, and denitrification to finally turn organic nitrogen that enters living systems via nitrogen fixation back into nitrogen gas. The

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ammonification process is the initial stage in terrestrial systems, when some bacteria and fungi turn nitrogenous waste from live species or from the remnants of dead ones into ammonia (NH3). Through the process of nitrification, nitrifying soil bacteria such as those in the genus Nitrosomonas convert this ammonia to nitrite (NO2), then to nitrate (NO3). Last but not least, nitrate is transformed into nitrogen gas that reenters the atmosphere through the process of denitrification, which involves soil bacteria like those from the genera Pseudomonas and Clostridium using nitrate as a terminal electron acceptor in anaerobic respiration. Similar activities take place in the marine nitrogen cycle, where marine bacteria and archaea carry out these three processes.

The usage of synthetic fertilisers that include nitrogen and phosphorus compounds, which are subsequently washed into lakes, rivers, and streams via surface runoff, causes human activity to release nitrogen into the environment. The expansion and eventual death of aquatic algae due to fertiliser runoff results in saltwater and freshwater eutrophication, which renders water sources anaerobic and unsuitable for the existence of aquatic species.

Nitrogen Cycle

Gaseous atmospheric nitrogen is absorbed by bacteria and lightning to transform into organic matter ($R\text{-}NH_2$). Ammonium (NH_{4+}) is created through mineralization and fertilisers. Runoff and leaching are two ways that this might get into rivers. Through nitrification, ammonium is transformed into nitrates (NO_{2-}). These are then nitrified and turned into nitrates (NO_{3-}). Both of these may cause eutrophication in streams. Plants may absorb nitrates or denitrify them to produce gaseous nitrate (N_2).

Sulfur Cycle

Sulfur is a crucial component of the macromolecules found in living things. It participates in the synthesis of proteins together with the amino acid's cysteine and methionine. Additionally, it is included in a number of vitamins that are essential for the creation of significant biological components like coenzyme A. The activities involved in the sulphur cycle are carried out by many kinds of microorganisms (Figure 8.26). In order to use hydrogen sulphide as an electron donor, chemoautotrophic archaea and bacteria first oxidise it to elemental sulphur (S_0), then to sulphate (S_2O_4).

As a result, hydrogen sulphide levels in soil stratify, rising at deeper, more anaerobic depths. Sulfate can be used as a sulphur source by a wide variety of bacteria and plants. By removing sulphur groups from amino acids during the decomposition of dead organisms, fungi and bacteria create hydrogen sulphide, which releases inorganic sulphur back into the environment.

Plants absorb atmospheric sulphur (SO_2 gas). Organic sulphur is produced by plant waste, animal manure, and biosolids. Sulfate is produced by mineralization (S_2O_{4-}). Sulfate transforms back into organic sulphur during immobilisation. Anaerobic respiration transforms sulphate into H_2S. H_2S is also a product of decomposition.

Mineral sulphur may be produced or absorbed from sulphate. Sulfate is transformed into reduced sulphur via bacterial reduction (H_2S, HS). Sulfate is produced through the oxidation of reduced sulphur and elemental sulphur (SO_0). The sulphur cycle is shown in this image. It should be noted that certain prokaryotic groups may take part in each stage of the cycle independently. (Credit: NOAA modified this work.)

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A Different Biogeochemical Cycle

Prokaryotes participate in a variety of biogeochemical cycles in addition to the carbon, nitrogen, and sulphur cycles. Several of these additional biogeochemical cycles, including the iron (Fe), manganese (Mn), and chromium (Cr) cycles, involve redox chemistry, with prokaryotes participating in both oxidation and reduction. This is similar to the carbon, nitrogen, and sulphur cycles. Other elements go through chemical processes without using redox chemistry. These include the cycles of phosphorus (P), calcium (Ca), and silica (Si). Because significant amounts of these elements are incorporated into the exoskeletons of marine organisms, the cycling of these elements is particularly crucial in oceans. These biogeochemical cycles involve variations in the solubility of compounds containing calcium, phosphorous, and silica rather than redox chemistry. The availability of nitrogen (as was already mentioned), phosphorus, and iron typically serves as a barrier to the overgrowth of naturally occurring microbial communities. Eutrophication may result from human activities that introduce high levels of iron, nitrogen, or phosphorus (usually from detergents).

Bioremediation

Utilizing microbial metabolism, microbial bioremediation eliminates xenobiotics or other contaminants. Compounds known as xenobiotics are ones that humans have produced and released into the environment at levels much beyond those that would naturally occur. Adhesives, dyes, flame retardants, lubricants, oil and petroleum products, organic solvents, insecticides, and byproducts of the combustion of gasoline and oil are examples of environmental contaminants. Many xenobiotics are resistant to being broken down, and some of them accumulate in the food chain after being eaten or absorbed by fish and other animals that may then be ingested by humans. Contaminants including trichloroethylene (TCE), a frequent groundwater pollutant, and polycyclic aromatic hydrocarbon (PAH), a carcinogenic xenobiotic found in crude oil, are of special concern. There are two types of bioremediation processes: in situ and ex situ. In situ bioremediation, which takes place at the site of pollution, does not entail moving contaminated material. Ex situ bioremediation, in contrast, is the removal of contaminated material from the original site so that it may be treated elsewhere. Typically, this treatment takes place in a large, lined pit where the environment is ideal for the contaminant's destruction.

Some bioremediation techniques depend on microbes that are naturally occurring in the polluted area or substance. Enhanced bioremediation methods, which may be used for in situ or ex situ processing, provide nutrients, air, or both to promote the development of bacteria that break down pollutants. They may also introduce non-native microbes with a reputation for breaking down toxins. For instance, many *Rhodococcus* and *Pseudomonas* bacteria are well recognised for their capacity to convert numerous environmental pollutants, such as aromatic compounds like those present in oil, to CO_2. On plasmids, the genes for their degradative enzymes are often identified. Some microorganisms, such as *Alcanivorax borkumensis*, create surfactants that are helpful in solubilizing the hydrophobic compounds present in oil, hence increasing their accessibility to other microbes for breakdown.

Although a DNA test specifically for *Neisseria meningitidis* exists, it is impractical for application in certain poor nations due to the high cost of the necessary tools and the degree of skill needed to execute it. DNA testing could not be done at the hospital in Banjul. However, biochemical testing is far less costly and is still useful for identifying microorganisms. Fortunately for Hannah, taking antibiotics helped her symptoms start to go away. After many weeks of recuperation, Hannah did

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not seem to be demonstrating any long-term symptoms, and her behaviour had returned to normal. Patients who survive bacterial meningitis often have long-term issues include brain damage, hearing loss, and seizures. Her parents were urged to constantly watch for any indications of developmental problems due to her age and to have frequent doctor evaluations. 10%–20% of people have *N. meningitidis* in their typical respiratory microbiome. Although the bacteria often do not cause illness, it may sometimes enter the circulation and infect other parts of the body, including the brain, for unknown reasons. Like Hannah, the condition is most prevalent among young children and newborns.

The so-called "meningitis belt," an area of sub-Saharan Africa that spans 26 nations from Senegal to Ethiopia, has a particularly high frequency of meningitis caused by *N. meningitidis* (Figure 8.27). Although the causes of this high frequency are unclear, a number of variables, including the dry, dusty environment, overcrowding, and subpar living conditions, as well as the population's generally poor nutritional and immunocompetence, may be involved in greater rates of transmission. 3 There is a vaccination available to protect against four types of *N. meningitidis* bacteria. Children between the ages of 11 and 12 should be vaccinated, with a booster shot suggested at age 16. In addition, vaccination is advised for young individuals who reside in settings where the illness is more readily spread (such as military barracks or student residence halls). Visitors to the "meningitis belt" should also be immunised, particularly from December through June, when the frequency is at its maximum.

Despite the planet's estimated age of 4.6 billion years, there wasn't enough oxygen in the atmosphere for life as we know it to exist for the first 2 billion years. One explanation for the origin of life on Earth is the notion of a "primordial soup." In accordance with this idea, the metals and gases from the atmosphere combined with an energy source, such as lightning or UV radiation, in a body of water to produce the carbon compounds that make up the chemical basis of life. In 1952, graduate student Stanley Miller (1930-2007) and his professor Harold Urey (1893-1981) at the University of Chicago attempted a now-famous experiment to test this notion. Miller and Urey combined water (H_2O), methane (CH_4), hydrogen (H_2), and ammonia (NH_3), then sealed the mixture in a sterile flask. These compounds were believed to be the primary components of the early atmosphere of the planet. They then heated the flask to produce water vapour, passed the combination through the flask, and employed electric sparks to mimic lightning in the sky. They found amino acids, which are the chemical building blocks of proteins and are essential to the existence of all living things, when they opened the flask to investigate its contents a week later.

With the aim of using chemical principles to explain form and function, the study of biochemistry investigates the chemistry of life. The area of organic chemistry is concerned with the study of carbon-based chemistry, which serves as the foundation for the study of biomolecules and the science of biochemistry. Both biochemistry and organic chemistry are built upon the general chemistry concepts.

Living cells' constituent parts

In contrast to carbon (C), oxygen (O), nitrogen (N), phosphorus (P), and sulphur (S), hydrogen (H) dominates in cells (S). These elements are referred to as macronutrients because they account for about 99% of the dry weight of cells. Some species need very little amounts of certain chemicals, sometimes referred to as micronutrients or trace elements. The following elements fall into this category: sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), calcium (Ca), molybdenum (Mo), copper (Cu), cobalt (Co), manganese (Mn), or vanadium (V). All of these

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elements are essential to the functioning of several biochemical processes and therefore to life. The four elements (C, N, O and H) that make up the bulk of organic matter are light elements with low atomic numbers that can join together to form molecules. In contrast to carbon, which generates four chemical bonds, nitrogen, oxygen, and hydrogen each produce three. One or more "lone pairs" of electrons, which are often present when oxygen, sulphur, and nitrogen are connected together within molecules, have a significant impact on the physical and chemical properties of many compounds. Due to these properties, a wide range of molecular species may be produced, which is necessary for the development of living creatures' structures and their ability to function.

There is the carbon atom in the centre of carbon dioxide (CO_2). This carbon atom is doubly connected to two oxygen atoms, one on the left and one on the right. Ammonia, or NH_3, is made up of three hydrogen atoms and one nitrogen atom. Oxygen is made up of two atoms (O_2) There is the carbon atom in the centre of carbon dioxide (CO_2). This carbon atom is doubly connected to two oxygen atoms, one on the left and one on the right. Ammonia, or NH3, is made up of three hydrogen atoms and one nitrogen atom. In oxygen, there is a doubly connected pair of oxygen atoms (O_2). ms are joined by a double bond. Carbon dioxide, ammonia, and oxygen are examples of common molecules, and they are composed of mixtures of oxygen, carbon, hydrogen, nitrogen, and/or hydrogen atoms (red spheres), as well as carbon and hydrogen atoms (grey spheres) (blue spheres).

Components of Living Cells

In cells, hydrogen (H) predominates over carbon (C), oxygen (O), nitrogen (N), phosphorous (P), and sulphur (S). These substances, which make up nearly 99% of the dry weight of cells, are known as macronutrients. Some substances, known as micronutrients or trace elements, are needed by some organisms in extremely minute quantities. These substances include sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), calcium (Ca), molybdenum (Mo), copper (Cu), cobalt (Co), manganese (Mn), or vanadium (V). These substances are all necessary for the operation of several biochemical processes and are hence necessary for life.

The four elements that make up the majority of organic stuff (C, N, O and H) are light elements with low atomic numbers that may create molecules by making tight connections with other atoms. Nitrogen, oxygen, and hydrogen each create three chemical bonds, whereas carbon forms four. The physical and chemical characteristics of many molecules are greatly influenced by the presence of one or more "lone pairs" of electrons, which are often present when oxygen, sulphur, and nitrogen are linked together inside molecules. These characteristics allow the production of a sizable variety of molecular species that are required to create the structures and enable the activities of living organisms.

The central carbon atom of carbon dioxide (CO_2) is present. Two oxygen atoms, one on the left and one on the right, are doubly linked to this carbon atom. Three hydrogen atoms and a nitrogen atom make up ammonia, or NH_3. Two oxygen atoms make up oxygen (O_2) The central carbon atom of carbon dioxide (CO_2) is present. Two oxygen atoms, one on the left and one on the right, are doubly linked to this carbon atom. Three hydrogen atoms and a nitrogen atom make up ammonia, or NH_3. Two oxygen atoms are doubly linked to one another in oxygen (O_2). double-bonded ms to one another. Common molecules include carbon dioxide, ammonia, and oxygen, which are made up of combinations of oxygen, carbon, hydrogen, and/or nitrogen atoms (red spheres), as well as carbon and hydrogen atoms (grey spheres) (blue spheres).

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Both inorganic substances (mostly water and salts; see Appendix A) and organic molecules are found in living things. Inorganic substances lack carbon whereas organic molecules do. The exceptions include carbon oxides and carbonates, which contain carbon but are categorised as inorganic because they lack hydrogen. An organic molecule's atoms are often arranged in chains around carbon atoms. Living cells contain 1%–1.5% of their dry weight in inorganic substances. Despite not contributing to the formation of cell structures, these little, simple molecules have significant functions in cells. The majority of the carbon that makes up organic molecules comes from inorganic sources, such as carbon dioxide that is taken in by microbes via carbon fixation.

Isomerism and Organic Molecules

In general, organic molecules in living things are bigger and more complex than inorganic ones. Covalent bonds keep their carbon skeletons attached to one another. They carry out the chemical processes necessary for life and build the cells of an organism. All of these molecules include carbon, which is the fundamental component of life and is referred to as a biomolecule since it is a component of living matter. Because it has four valence electrons in its outer orbitals and may simultaneously make four single covalent bonds with up to four other atoms, carbon is a particularly special element. These atoms often include carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorus. Methane is the most basic organic chemical, in which carbon solely interacts with hydrogen.

Due to the special size and bonding characteristics of carbon, a chain or carbon skeleton may be created when a significant number of carbon atoms are bound together. Organic molecules' carbon skeletons may be ring-shaped, branching, or straight (cyclic). Organic molecules are composed of chains of carbon atoms of various lengths; the majority are often relatively long, allowing for a very large number of compounds. No other element can create as many distinct molecules with such a wide range of sizes and forms.

Isomers are molecules having the same atomic make-up but a distinct atomic configuration. Chemistry places a great deal of emphasis on the idea of isomerism since molecules' structures are always intimately linked to their functions. Very varied qualities may result from little adjustments to the atoms' structural configurations inside molecules. In chemistry, molecules are represented by their structural formula, which is a diagram that illustrates the arrangement of the atoms inside the molecule. Structural isomers are substances with identical molecular formulae but different atom bonding arrangements. Although the monosaccharides fructose, galactose, and glucose all have the same chemical formula of $C_6H_{12}O_6$, demonstrates that the atoms are not all equally linked.

Enantiomers are a special category of stereoisomers, which are isomers that vary in the spatial arrangements of atoms. Louis Pasteur made the first discovery of enantiomers in 1848 while using a microscope to examine crystalline wine fermentation products. Enantiomers are molecules with the chirality property, which means that their structures are not mirror reflections of one another. As seen by the examples of structural variations in the enantiomeric forms of the monosaccharide glucose or the amino acid alanine, chirality is a crucial property in many physiologically significant compounds.

Many different kinds of chemicals can only be used by one enantiomeric form by some organisms as food sources and as the building blocks for cellular structures. When ingested as food, several enantiomeric amino acid forms have noticeably distinctive tastes and odours. For instance, D-aspartame has no taste, but L-aspartame, sometimes known as aspartame, has a sweet flavour. The

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pharmacologic effects of various drug enantiomers might vary greatly. For instance, the chemical methorphan has two enantiomers, one of which suppresses coughing (dextromethorphan), and the other of which serves as an analgesic (levomethorphan, a drug similar in effect to codeine).

Due to their ability to change the direction of polarised light, enantiomers are also known as optical isomers. Light rotated clockwise in some of the crystals that Pasteur saw during the wine fermentation process, while it turned anticlockwise in others. Enantiomers that spin polarised light clockwise (+) are referred to as d forms, while its mirror counterpart, which rotates polarised light anticlockwise (), is referred to as the l form. The Latin words dexter (on the right) and laevus (on the left) are the origins of the letters d and l, respectively. The biological characteristics and actions of these two distinct optical isomers can vary greatly. Some mould, yeast, and bacterial species, including Rhizopus, Yarrowia, and Lactobacillus spp., can only break down one kind of optical isomer; the other isomer is not acceptable as a food source. Because certain microbes can only be affected by one particular optical isomer, therapeutic uses of these kinds of compounds for pharmacological therapy are another crucial reason to be aware of optical isomers.

Macromolecules

The majority of organic compounds are skeletonized by carbon chains. To create biomolecules, functional groups are combined with the chain. We refer to these biomolecules as macromolecules since they are often big in size. A large number of identical or extremely similar tiny organic macromolecules are linked together to create many physiologically significant macromolecules. The smaller molecules, known as monomers, serve as the basis for larger molecules, known as polymers, which are created when they are linked. Polysaccharides, proteins, lipids, and nucleic acids are the four primary classes of carbon-containing macromolecules found in cells and cell structures. This chapter will focus on the first three classes of molecules. In Biochemistry of the Genome, nucleic acids' biochemistry will be covered.

Carbohydrates are the most prevalent biomolecules in the world. Carbohydrates are essentially composed of carbon and water from a chemical perspective, and many of them have the empirical formula $(CH_2O)_n$, where n is the number of repeating units. According to this perspective, these molecules are simply represented as chains of carbon atoms that have had water molecules attached to them, giving rise to the word "carbohydrates." All carbohydrates have the elements carbon, hydrogen, and oxygen, but some additionally include the elements nitrogen, phosphorus, and/or sulphur. Carbohydrates serve a variety of purposes. They are numerous in terrestrial habitats, and humans eat a variety of their species. Additionally, these molecules play a crucial role in the macromolecular systems that carry and store genetic information (i.e., DNA and RNA). They serve as the building blocks for biological polymers that provide organisms' structural parts strength (such cellulose and chitin), and they are the main source of the energy storage molecules starch and glycogen.

The Sweet Ones of the Monosaccharide's

Although not all carbohydrates are sweet, they are often referred to as saccharides in biochemistry, from the Greek sakcharon, which means sugar. Simple sugars, often known as monosaccharides, are the most basic kind of carbohydrates. As will be covered later in this section, they serve as the building blocks (monomers) for the production of polymers or complex polysaccharides. Based on how many carbons are present in the molecule, monosaccharides are categorised. Prefixes that denote the number of carbons and the suffix -ose that denotes a saccharide are used to identify

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general groups. Examples include triose (three carbons), tetrose (four carbons), pentose (five carbons), and hexose (six carbons). The most prevalent monosaccharide in nature is hexose D-glucose. Galactose, which is utilised to create the disaccharide milk sugar lactose, and fructose, a sugar found in fruits, are two other extremely prevalent and frequent hexose monosaccharides. When monosaccharides with four or more carbon atoms adopt cyclic, or ring, forms, they are often more stable. The carbonyl group and a relatively far-off hydroxyl group, which are functional groups on opposing ends of the sugar's flexible carbon chain, combine chemically to form these ring formations. For instance, glucose forms a six-membered ring.

Disaccharides

A disaccharide may be created by the chemical bonding of two monosaccharide molecules. A glycosidic bond is the term used to describe the covalent connection between the two monosaccharides.

Polysaccharides

Large polymers made of hundreds of monosaccharide monomers are known as polysaccharides, sometimes known as glycans. Polysaccharides, in contrast to mono- and disaccharides, are not sweet, and they typically are not soluble in water. The monomeric units of polysaccharides are joined by glycosidic linkages, much as disaccharides. Polysaccharides have a very wide range of structural variations. Starch, glycogen, and cellulose, three of the most significant polysaccharides in biology, are all made up of repeated glucose units despite having different structures. Cellulose is a common structural element of plant and other organism cell walls and is made up of a linear chain of glucose molecules. Both starch and glycogen are branching polymers; starch is the principal energy-storage molecule in plants, while glycogen is the primary energy-storage molecule in animals and microorganisms. Linear and branching macromolecules have varied characteristics as a result of the varying glycosidic bond orientations in these three polymers.

Other structural polysaccharides may have modified glucose molecules as their core molecules. These structural polysaccharides include N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM), which are present in the peptidoglycan of bacterial cell walls. Chitin is a polymer of NAG that is present in insect exoskeletons and fungal cell walls. Lipid molecules may include other elements than carbon and hydrogen, such as oxygen, nitrogen, sulphur, and phosphorus. Lipids have a variety of vital roles in the structure and operation of organisms. They may serve as a source of nutrition, a container for carbon, molecules that store energy, or even the building blocks of hormones and membranes. The most prevalent lipids—a large class of chemically diverse compounds—are covered in this section.

Triacylglycerides and Fatty Acids

The fatty acids are lipids with long-chain hydrocarbons that have carboxylic acid functional groups at the end of their chains. Fatty acids are hydrophobic ("hating water") or nonpolar due to the lengthy hydrocarbon chain. Because they have the maximum amount of hydrogen atoms possible and are therefore "saturated" with hydrogen, fatty acids with hydrocarbon chains that only have single bonds are known as saturated fatty acids. Because they have fewer hydrogen atoms, fatty acids with hydrocarbon chains having at least one double bond are known as unsaturated fatty acids. Unsaturated fatty acids feature "kinks" in their carbon skeleton because each double bond results in a hard bend of the carbon skeleton, in contrast to saturated fatty acids, which have a

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straight, flexible carbon backbone. The related lipids in which the fatty acids are integrated have various characteristics as a consequence of these variations in saturated vs unsaturated fatty acid structure. For instance, unsaturated fatty acid-containing lipids are liquids at normal temperature whereas saturated fatty acid-containing lipids are solids.

When three fatty acids are chemically joined to a glycerol molecule, a triacylglycerol, also known as a triglyceride, is created. Triglycerides make up a large portion of sebum and are the main building blocks of adipose tissue (body fat) (skin oils). They serve a significant metabolic function by acting as effective energy-storage molecules that may provide two to three times as many calories as proteins and carbs.

Biological Membranes and Phospholipids

Triglycerides are categorised as simple lipids since they only include glycerol and fatty acids as constituents. Complex lipids, on the other hand, include at least one extra component, such as a phosphate group (phospholipids) or a carbohydrate moiety (glycolipids). One, both, or none of the two fatty acid carbon chains may be saturated. The third binding location on the glycerol molecule is filled by a modified phosphate group rather than a different fatty acid molecule (as is the case with triglycerides).

Lipids' molecular makeup causes them to behave differently in watery conditions. These substances are nonpolar and weakly attracted to polar water molecules because all three of the substituents on the glycerol backbone are long hydrocarbon chains; they are hydrophobic. On the other hand, phospholipids contain a negatively charged phosphate group, such as the one in Figure 7.13. The phosphate is charged, which enables it to bind water molecules strongly, making it hydrophilic, or "loving water." Long hydrocarbon chains are often referred to as nonpolar "tails," whereas the hydrophilic section of the phospholipid is sometimes referred to as a polar "head." Amphipathic describes a molecule with a hydrophobic and a hydrophilic moiety. Examples of lipids with carbohydrates attached to their head groups include glycolipids.

Phospholipids' amphipathic properties allow them to form specifically useful structures in watery conditions. As previously established, these molecules' nonpolar tails are not attracted to water molecules as strongly as their polar heads are. These tails are really quite attracted to one another because of their enormous lengths. As a consequence, large-scale assemblies of phospholipid molecules that are energetically stable are created, in which the polar heads protect the hydrophobic tails from water contact and the hydrophobic tails collect in enclosed areas. The most basic of these structures are micelles, which are spherical assemblies with a hydrophobic core made up of phospholipid tails and a polar head group surface. Lipid-bilayer sheets, or unit membranes, are huge, two-dimensional assemblages of phospholipids gathered tail to tail. They are used to build larger and more sophisticated structures. The membranes of numerous internal components as well as the cell membranes of almost all organisms are constructed from lipid-bilayer sheets. These sheets may also result in the formation of lipid-bilayer spheres, which serve as the structural underpinning for vesicles and liposomes, subcellular components involved in a variety of physiological processes.

Sterols and Isoprenoids

The branching lipids known as isoprenoids, which are also known as terpenoids, are created by chemically altering the isoprene molecule. These lipids serve a variety of physiological roles in

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both plants and animals in addition to their many industrial uses as fragrances, colours, and smells (such as orange beta carotene and xanthophylls) and medicines (like capsaicin) (e.g., menthol, camphor, limonene [lemon fragrance], and pinene [pine fragrance]). Long-chain isoprenoids are also present in hydrophobic oils and waxes. Waxes are typically tough and water-resistant at room temperature, but when heated, they become softer and eventually liquefy. In the skin of humans, the sebaceous glands of hair follicles release sebum, a lubricant that mostly consists of triacylglycerol, wax esters, and the hydrocarbon squalene. There are various bacteria that feed on these lipids in the skin's microbiome. One of the most well-known lipid-eating bacteria, Propionibacterium acnes, uses the skin's lipids to make short-chain fatty acids, which is how acne develops.

An further category of lipids is called steroids; these complex, ringed molecules are found in cell membranes and may sometimes function as hormones. The most common kind of steroids are sterols, or those that have an OH group. These molecules are mostly hydrophobic, albeit they do include hydrophilic hydroxyl groups. In animal tissues, cholesterol predominates among other sterols. Its four rings, one of which contains a double bond, are composed of the hydroxyl group, which is situated where the sterol is defined. Cholesterol has the function of fortifying cell membranes in eukaryotes and bacteria without cell walls, such as Mycoplasma. Prokaryotes often lack the ability to produce cholesterol, in contrast to bacteria, which can produce hopanoids, related compounds with similar multiringed structures that support bacterial membranes. The cell membranes of these organisms are reinforced by ergosterol, a similar compound generated by a variety of protozoa and fungus.

The famous experiment that generated amino acids under conditions that mimicked those that existed on Earth long before the birth of life as we know it was covered in length at the beginning of this chapter. These substances have the capacity to join together in virtually any number, resulting in molecules of nearly any size with a wide range of physical and chemical properties and carrying out a variety of essential functions for all species. Aside from acting as building blocks for cells and subcellular structures, compounds produced from amino acids may also operate as food sources, atom and energy storage containers, and functional species including hormones, enzymes, receptors, and transport molecules.

Amino Acids and Peptide Bonds

An amino acid is an organic compound that contains one carbon atom, sometimes known as "carbon," together with two carboxyl groups (-COOH) and one amino group (-NH2). These groups, which are known as residues or side chains and are represented by the letter R in structural equations, are connected to the carbon atoms of various amino acids in different ways. A residue—also known as a monomer—is created when two or more amino acids combine and remove water molecules. Peptide chains, the basic unit of construction for proteins, are made up of amino acid residues. Because of the unique characteristics of the functional groups and R groups, these amino acid components may form hydrogen, ionic, and disulfide connections as well as the polar/nonpolar interactions necessary to produce secondary, tertiary, and quaternary protein structures. These groups are mostly composed of carbon, hydrogen, oxygen, nitrogen, and sulphur. They may be found in the forms of hydrocarbons, acids, amides, alcohols, and amines.

Amino acids may chemically bond by interacting with one other via their amine and carboxylic acid groups. The production of a peptide bond and a water molecule is the consequence of this procedure, another example of dehydration synthesis. Prefixes are often used to denote these

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numbers: dipeptides (two amino acids), tripeptides (three amino acids), and so on. Peptides are compounds that are generated by chemically combining a small number of amino acids (50 or fewer). Creating oligopeptides from a combination of up to 20 amino acids and polypeptides from a combination of up to 50 amino acids is a more generic way to describe the approximate number of amino acids. When a substantial number of amino acids are linked together or when several polypeptides are used as the building blocks, the resulting macromolecules are referred to as proteins. Because each amino acid has a variety of possible R groups and because these biopolymers' lengths (number of monomers) are constantly changing, the possible protein varieties are essentially limitless.

The size (length) and distinctive amino acid sequence of a protein have a significant impact on how it looks, and a protein's shape is crucial to how it works. For example, during the biological nitrogen fixation process (see Biogeochemical Cycles), soil bacteria collectively referred to as rhizobia interact symbiotically with the roots of legume plants such as soybeans, peanuts, or beans to create a new structure on the plant roots known as a nodule. The second transport protein produced by the plant is called leghemoglobin, which carries either oxygen or nitrogen. An area of the protein where the structure and amino acid sequence are appropriate for leghemoglobin to bind to its substrate oxygen with a very high affinity is that region (the active site). If the active site's chemical environment or form is even slightly altered, the substrate may not be able to bind as strongly or at all. Therefore, for a protein to be fully active, it has to have the appropriate structure for its activity.

Primary, secondary, tertiary, and quaternary are the four layers that make up protein structure. The order of amino acids that make up the polypeptide chain constitutes the fundamental structure. The nature of the bonds that hold the amino acids together makes the chain of amino acids that makes up a protein's main structure flexible rather than rigid. When the chain is long enough, hydrogen bonding between the amine and carbonyl functional groups in the peptide backbone (apart from the R side group) may take place. This causes the polypeptide chain to locally fold into helices and sheets. The secondary structure of a protein is made up of these forms. The -helix and the -pleated sheet are the two most prevalent secondary structures. The oxygen atom in a carbonyl group of one amino acid and the hydrogen atom of the amino group only four amino acid units farther down the chain form hydrogen bonds, which hold the helix together in the -helix structure. In the -pleated sheet, analogous hydrogen bonds are used to create the pleats between long continuous sequences of carbonyl and amino groups that are more spaced out on the polypeptide chain's backbone.

The tertiary structure, which is the massive three-dimensional form of a single polypeptide chain, is the next level of protein organisation. Interactions between amino acid residues located widely apart in the chain shape the tertiary structure. Protein tertiary structure is the result of several interactions, including hydrogen bonds, ionic connections, hydrophobic interactions between nonpolar side chains, and disulfide bridges, which are links between the sulfhydryl (-SH) functional groups on amino acid side groups. The ultimate three-dimensional form of the protein and its function are determined by the combination of all these weak and strong interactions.

Protein folding is the method through which a polypeptide chain takes on a substantial, three-dimensional form. The term "native structure" refers to a folded protein's whole biological functionality. A protein may cease to function if it loses its three-dimensional form. Denatured proteins are those that are unfolded. In the case of denaturation, the fundamental structure is retained but the secondary and tertiary structures (and, if present, the quaternary structure) are lost.

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Some proteins are collections of several distinct polypeptides, or protein subunits. Only when all subunits are present and properly arranged can these proteins work as intended. The quaternary structure of the protein is made up of the connections that keep these subunits together. Relatively weak contacts help to maintain the overall quaternary structure. For instance, haemoglobin has a quaternary structure composed of four globular protein subunits, each of which contains an iron-based heme.

A prominent subgroup of proteins are the conjugated proteins that include a nonprotein component. A glycoprotein is a protein that has been joined with a carbohydrate. If it has a lipid attached, it is referred to as a lipoprotein. The structure of the membrane is greatly influenced by these proteins. Accurate identification of bacterial isolates is essential in a clinical microbiology lab because the results often inform treatment decisions with a direct bearing on patient outcomes. For instance, physicians must precisely pinpoint the chemical that caused the issue in order to treat patients with food poisoning. Similar to this, in order to adopt successful containment measures for a disease outbreak, it is crucial to accurately identify the organism at fault.

There are several ways to locate, characterise, and identify microorganisms. Other methods rely on phenotypic biochemical features while others utilise genotypic identification. The biochemical characteristics of a bacteria provide a number of characteristics that are useful for classifying and identifying it. Examining a bacterium's nutritional and metabolic capabilities is a common way to determine its genus and species. Some of the most important metabolic processes that bacteria need to survive will be covered in Microbial Metabolism. We'll go through a couple methods in this section for recognising microorganisms based on their biochemical characteristics. It is possible to identify some microorganisms by the compounds that are stored within the granules that they maintain in their cytoplasm. For instance, certain Pseudomonas nonfluorescent bacteria have the carbon and energy storage molecule poly-hydroxybutyrate (PHB). Different species of this genus may be distinguished from one another based on the presence or absence of PHB and fluorescent pigments. The human pathogen P. aeruginosa and the plant pathogen P. syringae are two fluorescent Pseudomonas species that do not gather PHB granules.

Other methods for differentiating bacteria based on their biochemical activities, such as carbon consumption and other metabolic tests, rely on biochemical features. These tests are carried out in tiny lab settings or in educational facilities using a limited number of test tubes. Modern systems, however, depend on panels of biological processes that are run simultaneously and then analysed by software, such the one developed by Biolog, Inc. Based on physiological traits including pH and chemical sensitivity as well as their ability to metabolise certain biochemicals, the Biolog system can identify cells. Every key class of biochemicals is used in the research. Identifications may be carried either manually or with the use of partly or fully automated technology.

Another automated method identifies the specimen's mass spectrum and then compares it to a database of known mass spectra for thousands of microorganisms. This method, which is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and uses disposable MALDI plates, combines the microbe with a specific matrix reagent. The sample/reagent combination is exposed to a high-intensity pulsed ultraviolet laser, which triggers the ejection of gaseous ions created by the various chemical components of the microorganism. When these gaseous ions are collected and accelerated, their mass-to-charge ratio (m/z), which controls how rapidly they go through the mass spectrometer, determines when they reach the detector. A plot of detector signal versus m/z may be used to determine the mass spectrum for the

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organism, which is directly related to its metabolic composition. By comparing the mass spectrum to a database of reference spectra produced from analogous examinations of known microorganisms, it is feasible to identify the unknown bacterium.

Another way of identification involves analysing the unique lipid profiles of microorganisms. As we have learned, the chain length, number of double bonds, hydroxyl groups, branches, and rings, as well as whether or not double bonds are present, may all vary among fatty acids in lipids. In order to identify a microorganism based on its lipid composition, the fatty acids present in its membranes are analysed. A typical biochemical analysis is utilised for this purpose and is a technique used in clinical, public health, and food laboratories. This technique, referred to as fatty acid methyl ester (FAME) analysis, is concerned with identifying unique changes in fatty acids. Gas chromatography is used to collect fatty acids from the membranes of microorganisms, alter them chemically to form volatile methyl esters, and then analyse the findings (GC). The resulting GC chromatogram is compared to reference chromatograms in a database that has details on hundreds of bacterial isolates in order to identify the unidentified bacterium.

Analyzing fatty acids from phospholipids (PLFAs) is a method comparable to this one for detecting bacteria. The bulk of membranes are composed of phospholipids, which may be saponified (hydrolyzed with alkali) to release fatty acids. The unknown bacterium may be identified by comparing its measured lipid profile to that of other known microbes after FAME analysis of the resulting fatty acid mixture. The proteins that bacteria produce when they grow in certain conditions within a human body may also be used to identify them. These kinds of distinguishing procedures are referred to as proteome analyses. Prior to being digested to create smaller peptide fragments for proteome analysis, high-pressure liquid chromatography (HPLC) is used to separate the pathogen's proteins. The original material's unknown bacteria may be located by comparing these identified peptides to those of other recognised microbes using mass spectrometry.

Microorganisms may also be recognised by their glycoproteins, which are carbohydrates bound to proteins that are present in their cell walls or plasma membranes. Antibodies and other proteins that bind carbohydrates may attach to specific carbohydrates on cell surfaces, causing the cells to congregate. Serological tests (such the Lancefield groups tests) are used to check for specific carbohydrates on the cell surface in order to identify certain *Streptococcus species*.

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Questionnaires

1) What is Microbial Technology?
2) What are the different branches of Microbiology?
3) What are the major breakthrough in the discipline of Microbiology?
4) What do you mean by microscopy?
5) What is the difference between compound and simple microscope?
6) What are the common components of microscope?
7) What are different microscopes and their functions?
8) What are the basic techniques of staining?
9) Elaborate on positive and negative staining technique.
10) What is gram stain and how it is important?
11) What are preservation techniques for microbial culture?
12) What is microbial nutrition?

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Recommended Books for Further Reading/ Reference Books:

1) Basic Techniques in Biochemistry and Molecular Biology 3rd Edition by R K Sharma and S P S Sangha
2) Fundamental Techniques in Microbiology 5th Edition by John
3) Advances in Applied Microbiology 1st Edition by Geoffrey Michael Gadd and Sima Sariaslani
4) Textbook of Environmental Microbiology 2nd Edition by Pradipta K Mohapatra
5) Environmental Microbiology and Biotechnology 1st Edition by D P Singh
6) Fundamental Agricultural Microbiology 2nd Edition by K R Aneja
7) Applied Dairy Microbiology, Second Edition (Food Science and Technology) by Elmer H Marth and James Steele
8) Encyclopaedia of Agricultural Microbiology 1st Edition by Dr J S Bohra
9) Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas (Microbiology Monographs) 1st Edition by Min-Tze Liong
10) Microbiology and Technology of Fermented Foods (Institute of Food Technologists Series) 1st Edition by Robert W Hutkins

Recommended Microbiology Textbooks for Further Studies:

a. Prescott's Microbiology (9th Edition) **Authors**: Christopher J.Woolverton, Joanne Willey, and Linda Sherwood.

b. Bailey & Scott's Diagnostic Microbiology (13th Edition) *Author*: Patricia Tille.

c. Microbiology: An Introduction (12th Edition) *Authors*: Berdell R.Funke, Gerard J.Tortora, and Christine L.Case.

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