Bacteriology (MCB 301)

Course Details

1. Classification and Characterization of bacteria and eukaryotes

-morphology, lifecycle and biochemical characteristics. (Duration:2weeks)

2.Significant role of bacteria

-Agriculture, Industry, Pharmacy, Food, Environment.(Duration:1 week)

3.Bacterial infection and Methods in diagnostic bacteriology.(Duration 2 weeks)

4.Aspects of molecular bacteriology.(Duration:1 week)

5. Practicals in cultivation and physiology.(Duration:3 practical claseses)

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An understanding of the fundamentals of bacteriology is critical to bacteriologists and other forensic investigators attempting to identify potential biogenic pathogens that may be exploited as agents in biological warfare or by bioterrorists.

Fundamentals of Bacteriology

Bacteria are one-celled prokaryotic organisms that lack a true nucleus (i.e., a nucleus defined by a membrane). Bacteria maintain their genetic material, deoxyribonucleic acid (DNA), in a single, circular chain. Bacteria also contain DNA in small circular molecules termed plasmids. The Dutch merchant and amateur scientist Anton van Leeuwenhoek was the first to observe bacteria and other microorganisms. In addition to not being contained in a membrane bound nucleus, the DNA of prokaryotes is not associated with the special chromosome proteins called histones, which are found in higher organisms. In addition, prokaryotic cells lack other membrane-bounded organelles, such as mitochondria.

Although all bacteria share certain structural, genetic, and metabolic characteristics, important biochemical differences exist among the many species of bacteria. The cytoplasm of all bacteria is enclosed within a cell membrane surrounded by a rigid cell wall whose polymers, with few exceptions, include peptidoglycans—large, structural molecules made of protein carbohydrate. Bacteria also secrete a viscous, gelatinous polymer (called the glycocalyx) on their cell surfaces. This polymer, composed either of polysaccharide

,polypeptide, or both, is called a capsule when it occurs as an organized layer firmly attached to the cell wall. Capsules increase the disease-causing ability (virulence) of bacteria by inhibiting immune system cells called phagocytes from engulfing them.

The shape of bacterial cells are classified as spherical (coccus), rodlike (bacillus), spiral (spirochete), helical (spirilla) and comma-shaped (vibrio). Many bacilli and vibrio bacteria have whiplike appendages (called flagella) protruding from the cell surface. Flagella are composed of tight, helical rotors made of chains of globular protein called flagellin, and act as tiny propellers, making the bacteria very mobile. On the surface of some bacteria are short, hairlike, proteinaceous projections that may arise at the ends of the cell or over the entire surface. These projections, called fimbriae, facilitate bacteria adherence to surfaces.

Other proteinaceous projections, called pili, occur singly or in pairs, and join pairs of bacteria together, facilitating transfer of DNA between them. During periods of harsh environmental conditions some bacteria can produce within themselves a dehydrated, thick-walled endospore. These endospores can survive extreme temperatures, dryness, and exposure to many toxic chemicals and to radiation. Endospores can remain dormant for long periods (hundreds of years in some cases) before being reactivated by the return of favorable conditions.

Identification and Classification

The identification schemes of *Bergey's Manual* are based on morphology (e.g., coccus, bacillus), staining (gram-positive or negative), cell wall composition (e.g., presence or absence of peptidoglycan), oxygen requirements (e.g., aerobic, facultatively anaerobic) and biochemical tests (e.g., in which sugars are aerobically metabolized or fermented). Another important identification technique is based on the principles of antigenicity—the ability to stimulate the formation of antibodies by the immune system. Commercially available solutions of antibodies against specific bacteria (antisera) are used to identify unknown organisms in a procedure called a slide agglutination test. A sample of unknown bacteria in a drop of saline is mixed with antisera that has been raised against a known species of bacteria. If the antisera causes the unknown bacteria to clump (agglutinate), then the test positively identifies the bacteria as being identical to that against which the anti-sera was raised. The test can also be used to distinguish between strains, slightly different bacteria belonging to the same species. Pathogens are disease-causing bacteria that release toxins or poisons that interfere with some function of the host's body.

Aerobic and anaerobic bacteria. Oxygen may or may not be a requirement for a particular species of bacteria, depending on the type of metabolism used to extract energy from food (aerobic or anaerobic). Obligate aerobes must have oxygen in order to live. Facultative aerobes can exist in the absence of oxygen by using fermentation or anaerobic respiration. Anaerobic respiration and fermentation occur in the absence of oxygen, and produce substantially less ATP than aerobic respiration.

During the 1860s, the French microbiologist Louis Pasteur studied fermenting bacteria. He demonstrated that fermenting bacteria could contaminate wine and beer during manufacturing, turning the alcohol produced by yeast into acetic acid (vinegar). Pasteur also showed that heating the beer and wine to kill the bacteria preserved the flavor of these beverages a process of heating, now called pasteurization is still used to kill bacteria in some

alcoholic beverages, as well as milk. Pasteur described the spoilage by bacteria of alcohol during fermentation as being a "disease" of wine and beer. His work was thus vital to the later idea that human diseases could also be caused by microorganisms and that heating can destroy them.

Bacterial Growth

A population of bacteria in a liquid medium is referred to as a culture. In the laboratory, where growth conditions of temperature, light intensity, and nutrients can be made ideal for the bacteria, measurements of the number of living bacteria typically reveals four stages, or phases, of growth, with respect to time. Initially, the number of bacteria in the population is low. Often the bacteria are also adapting to the environment. This represents the lag phase of growth. Depending on the health of the bacteria, the lag phase may be short or long. The latter occurs if the bacteria are damaged or have just been recovered from deep-freeze storage.

After the lag phase, the numbers of living bacteria rapidly increases. Typically, the increase is exponential. That is, the population keeps doubling in number at the same rate. This is called the log or logarithmic phase of culture growth, and is the time when the bacteria are growing and dividing at their maximum speed.

The explosive growth of bacteria cannot continue forever in the closed conditions of a flask of growth medium. Nutrients begin to become depleted, the amount of oxygen becomes reduced, and the pH changes, and toxic waste products of metabolic activity begin to accumulate. The bacteria respond to these changes in a variety of ways to do with their structure and activity of genes. With respect to bacteria numbers, the increase in the population stops and the number of living bacteria plateaus. This plateau period is called the stationary phase. Here, the number of bacteria growing and dividing is equaled by the number of bacteria that are dying.

Finally, as conditions in the culture continue to deteriorate, the proportion of the population that is dying becomes dominant. The number of living bacteria declines sharply over time in what is called the death or decline phase.

Culturing of bacteria is possible such that fresh growth medium can be added at a rate equal to the rate at which culture is removed. The rate at which the bacteria grow is dependent on the rate of addition of the fresh medium. Bacteria can be tailored to grow relatively slow or fast and, if the set-up is carefully maintained, can be maintained for a long time. Bacterial growth requires the presence of environmental factors. For example, if a bacterium uses organic carbon for energy and structure (chemoheterotrophic bacteria) then sources of carbon are needed. Such sources include simple sugars (glucose and fructose are two examples). Nitrogen is needed to make amino acids, proteins, lipids and other components. Sulphur and phosphorus are also needed for the manufacture of bacterial components. Other elements, such as potassium, calcium, magnesium, iron, manganese, cobalt and zinc are necessary for the functioning of enzymes and other processes.

Bacterial growth is also often sensitive to temperature. Depending on the species, bacteria exhibit a usually limited range in temperatures in which they can growth and reproduce. For example, bacteria known as mesophiles prefer temperatures from 20°–50° C (68°–122° F).

Outside this range, growth and even survival is limited. Other factors, which vary depending on species, required for growth include oxygen level, pH, osmotic pressure, light and moisture.

Aspects of molecular bacteriology

Bacteria can exchange genetic material via conjugation. Genetic recombination between bacteria (or protists) occurs via a cytoplasmic bridge between the organisms. A primitive form of exchange of genetic material between bacteria involving plasmids also can occur. Plasmids are small, circular, extrachromosomal DNA molecules that are capable of replication and are known to be capable of transferring genes among bacteria. For example, resistance plasmids carry genes for resistance to antibiotics from one bacterium to another, while other plasmids carry genes that confer pathogenicity. In addition, the transfer of genes via bacteriophages—viruses that specifically parasitize bacteria—also serves as a means of genetic recombination.

Bioengineering uses sophisticated techniques to purposely transfer DNA from one organism to another in order to give the second organism new characteristics. For example, in a process called transformation, antibiotic susceptible bacteria that are induced to absorb manipulated plasmids placed in their environment can acquire resistance to that antibiotic substance due to the new genes they have incorporated. Similarly, in a process called transfection, specially constructed viruses are used to artificially inject bioengineered DNA into bacteria, giving infected cells some new characteristic.

Bacterial adaptation and resistance. Evolution has driven both bacterial diversity and bacterial adaptation. Some alterations are reversible, disappearing when the particular pressure is lifted. Other alterations are maintained and can even be passed on to succeeding generations of bacteria. The first antibiotic was discovered in 1929. Since then, a myriad of naturally occurring and chemically synthesized antibiotics have been used to control bacteria. Introduction of an antibiotic is frequently followed by the development of resistance to the agent. Resistance is an example of the adaptation of the bacteria to the antibacterial agent.

Antibiotic resistance can develop swiftly. For example, resistance to penicillin (the first antibiotic discovered) was recognized almost immediately after introduction of the drug. As of the mid 1990s, almost 80% of all strains of *Staphylococcus aureus* were resistant to penicillin. Meanwhile, other bacteria remain susceptible to penicillin. An example is provided by Group A *Streptococcus pyogenes* , another Gram-positive bacteria.

The adaptation of bacteria to an antibacterial agent such as an antibiotic can occur in two ways. The first method is known as inherent (or natural) resistance. Gramnegative bacteria are often naturally resistant to penicillin, for example. This is because these bacteria have another outer membrane, which makes the penetration of penicillin to its target more difficult. Sometimes when bacteria acquire resistance to an antibacterial agent, the cause is a membrane alteration that has made the passage of the molecule into the cell more difficult.

The second category of adaptive resistance is called acquired resistance. This resistance is almost always due to a change in the genetic make-up of the bacterial genome. Acquired resistance can occur because of mutation or as a response by the bacteria to the selective pressure imposed by the antibacterial agent. Once the genetic alteration that confers

resistance is present, it can be passed on to subsequent generations. Acquired adaptation and resistance of bacteria to some clinically important antibiotics became a great problem in the last decade of the twentieth century.

Bacteria adapt to other environmental conditions as well. These include adaptations to changes in temperature, pH, concentrations of ions such as sodium, and the nature of the surrounding support. This adaptation is under tight genetic control, involving the expression of multiple genes. Bacteria react to a sudden change in their environment by expressing or repressing the expression of a whole lost of genes. This response changes the properties of both the interior of the organism and its surface chemistry.

Another adaptation exhibited by a great many bacteria is the formation of adherent populations on solid surfaces. This mode of growth is called a biofilm; bacteria within a biofilm and bacteria found in other niches, such as in a wound where oxygen is limited, grow and divide at a far slower speed than the bacteria found in the test tube in the laboratory. Such bacteria are able to adapt to the slower growth rate, once again by changing their chemistry and gene expression pattern. When presented with more nutrients, the bacteria can often very quickly resume the rapid growth and division rate of their test tube counterparts.

A further example of adaptation is the phenomenon of chemotaxis, whereby a bacterium can sense the chemical composition of the environment and either moves toward an attractive compound or shifts direction and moves away from a compound sensed as being detrimental. Chemotaxis is controlled by more than 40 genes that code for the production of components of the flagella that propel the bacterium along, for sensory receptor proteins in the membrane, and for components that are involved in signaling a bacterium to move toward or away from a compound.

Bacteriocidal and bacteriostatic treatment of bacteria. Bacteriocidal is a term that refers to the treatment of a bacterium such that the organism is killed. Bacteriostatic refers to a treatment that restricts the ability of the bacterium to grow.

Bacteriocidal methods include heat, filtration, radiation, and the exposure to chemicals. The use of heat is a very popular method of sterilization in a microbiology laboratory. The dry heat of an open flame incinerates microorganisms like bacteria, fungi and yeast. The moist heat of a device like an autoclave can cause deformation of the protein constituents of the microbe, as well as causing the microbial membranes to liquefy. The effect of heat depends on the time of exposure in addition to form of heat that is supplied. For example, in an autoclave that supplies a temperature of 121° F (49.4° C), an exposure time of 15 minutes is sufficient to kill the so-called vegetative form of bacteria. However, a bacterial spore can survive this heat treatment. More prolonged exposure to the heat is necessary to ensure that the spore will not germinate into a living bacteria after autoclaving. The relationship between the temperature and the time of exposure can be computed mathematically.

A specialized form of bacteriocidal heat treatment is called pasteurization after Louis Pasteur, the inventor of the process. Pasteurization achieves total killing of the bacterial population in fluids such as milk and fruit juices without changing the taste or visual appearance .

Exposure to electromagnetic radiation such as ultraviolet radiation is a direct means of killing bacteria. The energy of the radiation severs the strands of deoxyribonucleic acid in many locations throughout the bacterial genome. With only one exception, the damage is so severe

that repair is impossible. The exception is the radiation resistant bacterial genus called *Deinococcus* . This genus has the ability to piece together the fragments of DNA in their original order and enzymatic stitch the pieces into a functional whole.

Bacteriostatic agents prevent the growth of bacteria. Refrigeration can be bacteriostatic for those bacteria that cannot reproduce at such low temperatures. Sometimes a bacteriostatic state is advantageous as it allows for the long-term storage of bacteria. Ultra-low temperature freezing and lyophilization (the controlled removal of water from a sample) are means of preserving bacteria. Another bacteriocidal technique is the storage of bacteria in a solution that lacks nutrients, but which can keep the bacteria alive. Various buffers kept at refrigeration temperatures can keep bacteria alive for weeks.

Bacterial Infection

Bacteria can multiply and cause an infection in the bloodstream. The invasion of the bloodstream by the particular type of bacteria is referred to as a bacteremia. If the invading bacteria also release toxins into the bloodstream, the malady can also be called blood poisoning or septicemia. *Staphylococcus* and *Streptococcus* are typically associated with septicemia. The bloodstream is susceptible to invasion by bacteria that gain entry via a wound or abrasion in the protective skin overlay of the body, or as a result of another infection elsewhere in the body, or following the introduction of bacteria during a surgical procedure or via a needle during injection of a drug.

Depending on the identity of the infecting bacterium and on the physical state of the human host (primarily with respect to the efficiency of the immune system), bacteremic infections may not produce any symptoms. However, some infections do produce symptoms, ranging from an elevated temperature, as the immune system copes with the infection, to a spread of the infection to the heart (endocarditis or pericarditis) or the covering of nerve cells (meningitis). In more rare instances, a bacteremic infection can produce a condition known as septic shock. The latter occurs when the infection overwhelms the ability of the body's defense mechanisms to cope. Septic shock can be lethal.

Septicemic infections usually result from the spread of an established infection. Bacteremic (and septicemic) infections often arise from bacteria that are normal resident on the surface of the skin or internal surfaces, such as the intestinal tract epithelial cells. In their normal environments the bacteria are harmless and even can be beneficial. However, if they gain entry to other parts of the body, these so-called commensal bacteria can pose a health threat. The entry of these commensal bacteria into the bloodstream is a normal occurrence for most people. In the majority of people, however, the immune system is more than able to deal with the invaders. If the immune system is not functioning efficiently then the invading bacteria may be able to multiply and establish an infection. Examples of conditions that compromise the immune system are another illness (such as acquired immunodeficiency syndrome and certain types of cancer), certain medical treatments such as irradiation, and the abuse of drugs or alcohol.

Examples of bacteria that are most commonly associated with bacteremic infections are *Staphylococcus* , *Streptococcus* , *Pseudomonas* , *Haemophilus* , and *Escherichia coli* .

The generalized location of bacteremia produces generalized symptoms. These symptoms can include a fever, chills, pain in the abdomen, nausea with vomiting, and a general feeling of ill health. Not all these symptoms are present at the same time. The nonspecific nature of the symptoms may prevent a physician from suspecting bacteremia until the infection is more firmly established. Septic shock produces more drastic symptoms, including elevated rates of breathing and heartbeat, loss of consciousness and failure of organs throughout the body. The onset of septic shock can be rapid, so prompt medical attention is critical.

As with many other infections, bacteremic infections can be prevented by observance of proper hygienic procedures including hand washing, cleaning of wounds, and cleaning sites of injections to temporarily free the surface of living bacteria. The rate of bacteremic infections due to surgery is much less now than in the past, due to the advent of sterile surgical procedures, but is still a serious concern.

Bacterial infection does not always result in disease—even if a pathogen is virulent (able to cause disease). The steps of pathogenesis (the process of causing actual disease) can depend on a number of genetic and environmental factors. In some cases, pathogenic bacteria produce toxins released extracellularly (exotoxins) that migrate from the actual site of infection to cause damage to cells in other parts of the body.

Practicals
 The practical classes will involve aseptic technique, different staining techniques, basic technique in bacterial cultivation (pure culture), and bacterial population count.