Sterilization

Sterilization refers to any process that removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents like prions present in a specific surface, object or fluid, for example food or biological culture media.] Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic.

Foods

One of the first steps toward modernized sterilization was made by Nicolas Appert who discovered that thorough application of heat over a suitable period slowed the decay of foods and various liquids, preserving them for safe consumption for a longer time than was typical. Canning of foods is an extension of the same principle and has helped to reduce food borne illness ("food poisoning"). Other methods of sterilizing foods include food irradiation and high pressure (pascalization).[5] One process by which food is sterilized is heat treatment. Heat treatment ceases bacterial and enzyme activity which then leads to decreasing the chances of low quality foods while maintaining the life of non-perishable foods. Moist heat sterilization takes between 20 and 40 minutes, inversely proportional to the food temperature. The use of dry heat sterilization uses longer times of susceptibility that may last up to 2 hours and that use much higher temperatures compared to moist heat sterilization. These temperatures may range from 160 to 180 degrees Celsius.

Medicine and surgery

Joseph Lister was a pioneer of antiseptic surgery. Apparatus to sterilize surgical instruments, 1914-1918.

In general, surgical instruments and medications that enter an already aseptic part of the body (such as the bloodstream, or penetrating the skin) must be sterile. Examples of such instruments include scalpels, hypodermic needles, and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals. Most medical and surgical devices used in healthcare facilities are made of materials that are able to go under steam sterilization. Ethylene oxide gas has been used since the 1950s for heat- and moisture-sensitive medical devices. Within the past 15 years, a number of new, low-temperature sterilization systems (e.g., vaporized hydrogen peroxide, peracetic acid immersion, ozone) have been developed and are being used to sterilize medical devices. Steam sterilization is the most widely used and the most dependable. Steam sterilization is nontoxic, inexpensive, rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics.

Types of sterilisation - physical, chemical, filteration.

Physical Methods - Steam

A widely used method for heat sterilization is the autoclave, sometimes called a converter or steam sterilizer. Autoclaves use steam heated to 121–134 °C (250–273 °F) under pressure. To achieve sterility, the article is placed in a chamber and heated by injected steam until the article reaches a temperature and time setpoint. Almost all the air is removed from the chamber, because air is undesired in the moist heat sterilization process (this is one trait that differs from a typical pressure cooker used for food cooking) . A general cycle would be anywhere between 3 and 15 minutes, (depending on the generated heat) at 121 °C (250 °F) at 100 kPa (15 psi), which is sufficient to provide a sterility assurance level of 10−4 for a product with a bioburden of 106 and a D-value of 2.0 minutes. Following sterilization, liquids in a pressurized autoclave must be cooled slowly to avoid boiling over when the pressure is released. This may be achieved by gradually depressurizing the sterilization chamber and allowing liquids to evaporate under a negative pressure, while cooling the contents.

Proper autoclave treatment will inactivate all resistant bacterial spores in addition to fungi, bacteria, and viruses, but is not expected to eliminate all prions, which vary in their resistance. For prion elimination, various recommendations state 121–132 °C (250–270 °F) for 60 minutes or 134 °C (273 °F) for at least 18 minutes. Most autoclaves have meters and charts that record or display information, particularly temperature and pressure as a function of time. The information is checked to ensure that the conditions required for sterilization have been met. Indicator tape is often placed on the packages of products prior to autoclaving, and some packaging incorporates indicators. The indicator changes color when exposed to steam, providing a visual confirmation. For autoclaving, cleaning is critical. Extraneous biological matter or grime may shield organisms from steam penetration. Proper cleaning can be achieved through physical scrubbing, sonication, ultrasound, or pulsed air. Pressure cooking and canning is analogous to autoclaving, .Moist heat causes the destruction of microorganisms by denaturation of macromolecules, primarily proteins. This method is a faster process than dry heat sterilization.

Dry heat sterilization

Hot air oven

Dry heat was the first method of sterilization and is a longer process than moist heat sterilization. The destruction of microorganisms through the use of dry heat is a gradual phenomenon. With longer exposure to lethal temperatures, the number of killed microorganisms increases. Forced ventilation of hot air can be used to increase the rate at which heat is transferred to an organism and reduce the temperature and amount of time needed to achieve sterility. At higher temperatures, shorter exposure times are required to kill organisms. This can reduce heat-induced damage to food products. The standard setting for a hot air oven is at least two hours at 160 °C (320 °F). A rapid method heats air to 190 °C (374 °F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects. Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (e.g. it does not cause rusting of steel objects).

Flaming

Flaming is done to inoculation loops and straight-wires in microbiology labs for streaking. Leaving the loop in the flame of a Bunsen burner or alcohol burner until it glows red ensures that any infectious agent is inactivated. This is commonly used for small metal or glass objects, but not for large objects . However, during the initial heating, infectious material may be sprayed from the wire surface before it is killed, contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may

leave carbon or other residues on the object if the object is not heated enough. A variation on flaming is to dip the object in a 70% or more concentrated solution of ethanol, then briefly touch the object to a Bunsen burner flame. The ethanol will ignite and burn off rapidly, leaving less residue than a gas flame

Incineration

Incineration is a waste treatment process that involves the combustion of organic substances contained in waste materials. This method also burns any organism to ash. It is used to sterilize medical and other biohazardous waste before it is discarded with non-hazardous waste. Bacteria incinerators are mini furnaces that incinerate and kill off any microorganisms that may be on an inoculating loop or wire.

Tyndallization

Named after John Tyndall, Tyndallization[24] is an obsolete and lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, and then repeating the process a total of three to four times. The incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth, and will not sterilize non-nutritive substrates like water. Tyndallization is also ineffective against prions.

Glass bead sterilizers

Glass bead sterilizers work by heating glass beads to 250 °C (482 °F). Instruments are then quickly doused in these glass beads, which heat the object while physically scraping contaminants off their surface. Glass bead sterilizers were once a common sterilization method employed in dental offices as well as biological laboratories, but are not approved by the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) to be used as a sterilizers since 1997. They are still popular in European and Israeli dental practices.

Chemical sterilization

Chemicals are also used for sterilization. Heating provides a reliable way to rid objects of all transmissible agents, but it is not always appropriate if it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. In these situations chemicals, either in a gaseous or liquid form, can be used as sterilants. While the use of gas and liquid chemical sterilants avoids the problem of heat damage, users must ensure that the article to be sterilized is chemically compatible with the sterilant being used and that the sterilant is able to reach all surfaces that must be sterilized (typically cannot penetrate packaging). In addition, the use of chemical sterilants poses new challenges for workplace safety, as the properties that make chemicals effective sterilants usually make them harmful to humans. The procedure for removing sterilant residue from the sterilized materials varies depending on the chemical and process that is used.

Ethylene oxide

Ethylene oxide (EO, EtO) gas treatment is one of the common methods used to sterilize, pasteurize, or disinfect items because of its wide range of material compatibility. It is also used to process items

that are sensitive to processing with other methods, such as radiation (gamma, electron beam, X-ray), heat (moist or dry), or other chemicals. Ethylene oxide treatment is the most common chemical sterilization method, used for approximately 70% of total sterilizations, and for over 50% of all disposable medical devices. Ethylene oxide treatment is generally carried out between 30 and 60 °C (86 and 140 °F) with relative humidity above 30% and a gas concentration between 200 and 800 mg/l. Typically, the process lasts for several hours. Ethylene oxide is highly effective, as it penetrates all porous materials, and it can penetrate through some plastic materials and films. Ethylene oxide kills all known microorganisms, such as bacteria (including spores), viruses, and fungi (including yeasts and moulds), and is compatible with almost all materials even when repeatedly applied.

The most common EO processing method is the gas chamber method. To benefit from economies of scale, EO has traditionally been delivered by filling a large chamber with a combination of gaseous EO either as pure EO, or with other gases used as diluents; diluents include chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs), and carbon dioxide. Ethylene oxide is still widely used by medical device manufacturers. Since EO is explosive at concentrations above 3%, EO was traditionally supplied with an inert carrier gas, such as a CFC or HCFC.

Nitrogen dioxide

Nitrogen dioxide (NO_2) gas is a rapid and effective sterilant for use against a wide range of microorganisms, including common bacteria, viruses, and spores. The unique physical properties of NO_2 gas allow for sterilant dispersion in an enclosed environment at room temperature and atmospheric pressure. The mechanism for lethality is the degradation of DNA in the spore core through nitration of the phosphate backbone, which kills the exposed organism as it absorbs NO_2. This degradation occurs at even very low concentrations of the gas.NO_2 has a boiling point of 21 °C (70 °F) at sea level, which results in a relatively highly saturated vapour pressure at ambient temperature. Because of this, liquid NO_2 may be used as a convenient source for the sterilant gas. Liquid NO_2 is often referred to by the name of its dimer, dinitrogen tetroxide (N_2O_4). Additionally, the low levels of concentration required, coupled with the high vapour pressure, assures that no condensation occurs on the devices being sterilized. This means that no aeration of the devices is required immediately following the sterilization cycle. NO_2 is also less corrosive than other sterilant gases, and is compatible with most medical materials and adhesives.

Ozone

Ozone is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.Ozone offers many advantages as a sterilant gas; ozone is a very efficient sterilant because of its strong oxidizing properties , capable of destroying a wide range of pathogens, including prions, without the need for handling hazardous chemicals since the ozone is generated within the sterilizer from medical-grade oxygen. The high reactivity of ozone means that waste ozone can be destroyed by passing over a simple catalyst that reverts it to oxygen and ensures that the cycle time is relatively short. The disadvantage of using ozone is that the gas is very reactive and very hazardous. The NIOSH's immediately dangerous to life and health limit (IDLH) for ozone is 5 ppm, 160 times smaller than the 800 ppm IDLH for ethylene oxide. Monitors for determining workplace exposure to ozone are commercially available.

Glutaraldehyde and formaldehyde

Glutaraldehyde and formaldehyde solutions (also used as fixatives) are accepted liquid sterilizing agents, provided that the immersion time is sufficiently long. To kill all spores in a clear liquid can take up to 22 hours with glutaraldehyde and even longer with formaldehyde. The presence of solid particles may lengthen the required period or render the treatment ineffective. Sterilization of blocks of tissue can take much longer, due to the time required for the fixative to penetrate. Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has a short shelf-life (<2 weeks), and is expensive. Formaldehyde is less expensive and has a much longer shelf-life if some methanol is added to inhibit polymerization to paraformaldehyde, but is much more volatile. Formaldehyde is also used as a gaseous sterilizing agent; in this case, it is prepared on-site by depolymerization of solid paraformaldehyde. Many vaccines, such as the original Salk polio vaccine, are sterilized with formaldehyde.

Hydrogen peroxide

Hydrogen peroxide, in both liquid and as vaporized hydrogen peroxide (VHP), is another chemical sterilizing agent. Hydrogen peroxide is a strong oxidant, which allows it to destroy a wide range of pathogens. Hydrogen peroxide is used to sterilize heat- or temperature-sensitive articles, such as rigid endoscopes. In medical sterilization, hydrogen peroxide is used at higher concentrations, ranging from around 35% up to 90%. The biggest advantage of hydrogen peroxide as a sterilant is the short cycle time. Whereas the cycle time for ethylene oxide may be 10 to 15 hours, some modern hydrogen peroxide sterilizers have a cycle time as short as 28 minutes.Vaporized hydrogen peroxide (VHP) is used to sterilize large enclosed and sealed areas, such as entire rooms and aircraft interior.

Peracetic acid

Peracetic acid (0.2%) is a recognized sterilant by the FDA for use in sterilizing medical devices such as endoscopes.

Radiation sterilization

Sterilization can be achieved using electromagnetic radiation, such as Ultraviolet light, X-rays and gamma rays, or irradiation by subatomic particles such as by electron beams. Electromagnetic or particulate radiation can be energetic enough to ionize atoms or molecules (ionizing radiation), or less energetic (non-ionizing radiation).

Non-ionizing radiation sterilization

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful for sterilization of surfaces and some transparent objects. Many objects that are transparent to visible light absorb UV. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation, so that it is very difficult to remove). It also damages some plastics, such as polystyrene foam if exposed for prolonged periods of time.

Ionizing radiation sterilization

Gamma radiation is very penetrating, and is commonly used for sterilization of disposable medical equipment, such as syringes, needles, cannulas and IV sets, and food. It is emitted by a radioisotope, usually cobalt-60 (60Co) or caesium-137 (137Cs), which have photon energies of up to 1.3 and 0.66

MeV, respectively. Use of a radioisotope requires shielding for the safety of the operators while in use and in storage. With most designs, the radioisotope is lowered into a water-filled source storage pool, which absorbs radiation and allows maintenance personnel to enter the radiation shield. One variant keeps the radioisotope under water at all times and lowers the product to be irradiated in the water in hermetically-sealed bells; no further shielding is required for such designs. Other uncommonly used designs use dry storage, providing movable shields that reduce radiation levels in areas of the irradiation chamber. Electron beam processing is also commonly used for sterilization. Electron beams use an on-off technology and provide a much higher dosing rate than gamma or X-rays. Irradiation with X-rays, gamma rays, or electrons does not make materials radioactive, because the energy used is too low. Generally an energy of at least 10 MeV is needed to induce radioactivity in a material.[56] Neutrons and very high-energy particles can make materials radioactive, but have good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.

Filteration

Fluids that would be damaged by heat, irradiation or chemical sterilization, such as drug solution, can be sterilized by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and protein solutions in medicinal drug processing. A microfilter with pore size of usually 0.22 µm will effectively remove microorganisms. Some staphylococcal species have, however, been shown to be flexible enough to pass through 0.22 µm filters. Smaller pore sizes lower the flow rate, so in order to achieve higher total throughput or to avoid premature blockage, pre-filters might be used to protect small pore membrane filters. Membrane filters used in production processes are commonly made from materials such as mixed cellulose ester or polyethersulfone (PES). The filtration equipment and the filters themselves may be purchased as pre-sterilized disposable units in sealed packaging or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes.

Pure Culture techniques

Spread plate method

Spread plate technique is the method of isolation and enumeration of microorganisms in a mixed culture and distributing it evenly. The technique makes it easier to quantify bacteria in a solution.

Principle

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry and at room temperature so that the agar can absorb the bacteria more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

Spread Plate Technique – Procedure

Make a dilution series from a sample.

Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.

Dip the L-shaped glass spreader into alcohol.
Flame the glass spreader (hockey stick) over a Bunsen burner.

Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petridish underneath at the same time.

Incubate the plate at 37°C for 24 hours.

Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.

Uses of Spread Plate Technique

It is used for viable plate counts, in which the total number of colony forming units on a single plate is enumerated.

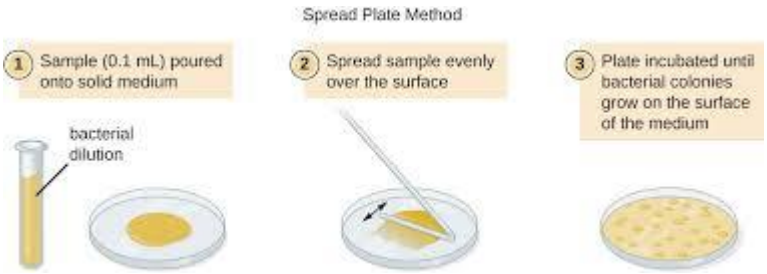
It is used to calculate the concentration of cells in the tube from which the sample was plated.

Spread plating is routinely used in enrichment, selection, and screening experiments.

Limitations of Spread Plate Technique

Strick aerobes are favored while microaerophilic tends to glow slower.

Crowding of the colonies makes the enumeration difficult.



Pour plate method

Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen. In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

Microorganisms will grow both on the surface and within the medium. Colonies that grow within the medium generally are small in size and may be confluent; the few that grow on the agar surface are of the same size and appearance as those on a streak plate. Each (both large and small) colony is carefully counted (using magnifying colony counter if needed). Each colony represents a "colony forming unit" (CFU).

The number of microorganisms present in the particular test sample is determined using the formula:

CFU/mL= CFU * dilution factor * 1/aliquot

For accurate counts, the optimum count should be within the range of 30-300 colonies/plate. To insure a countable plate a series of dilutions should be plated.pour-plate-technique-calculationThe pour plate method of counting bacteria is more precise than the streak plate method, but, on the average, it will give a lower count as heat sensitive microorganisms may die when they come contact with hot, molten agar medium.

Uses

The pour plate technique can be used to determine the number of microbes/mL in a specimen. It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of food stuffs.

Materials and Equipments

Test sample

Plate Count Agar (PCA) or Nutrient Agar

Hot water bath 45°C

Sterile Petri dishes

Flame

Colony counter with magnifying glass

Sterile capped 16*150 mm test tubes

Pipettes of various sizes (e.g. 01, 1.0 and 2.0 mL)

Procedure of Pour plate technique

Prepare the dilution of the test sample expected to contain between 30-300 CFU/mL. (Follow serial dilution technique)

Inoculate labeled empty petri dish with specified mL (0.1 or 1.0 mL) of diluted specimen

Pouring the molten agar and incubation

Collect one bottle of sterile molten agar (containing 15 mL of melted Plate Count Agar or any other standard culture media) from the water bath (45°C).

Hold the bottle in the right hand; remove the cap with the little finger of the left hand. Flame the neck of the bottle. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the the lid. Flame the neck of the bottle and replace the cap. Gently rotate the dish to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly. Do not slip the agar over the edge of the petri dish. Allow the agar to completely gel without disturbing it, it will take approximately 10 minutes. Seal and incubate the plate in an inverted position at 37°C for 24-48 hours.

Results

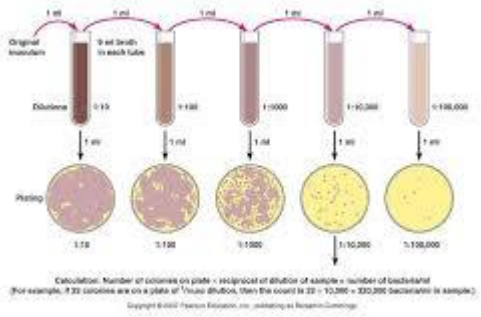
After 24-48 hours, count all the colonies (again: note that the embedded colonies will be much smaller than those which happen to form on the surface). A magnifying colony counter can aid in counting small embedded colonies.

Calculate CFU/mL using the formula: CFU/mL= CFU * dilution factor * 1/aliquot

(the volume of diluted specimen (aliquot) is either 0.1 or 1.0 mL)

Disadvantages of Pour plate method

Preparation for pour plate method is time consuming compared with streak plate/and or spread plate technique. Loss of viability of heat-sensitive organisms coming into contact with hot agar. Embedded colonies are much smaller than those which happen to be on the surface. Thus, one must be careful to score these so that none are overlooked. Reduced growth rate of obligate aerobes in the depth of the agar.



Streak plate method

Streak plate technique is used for the isolation into a pure culture of the organisms (mostly bacteria), from a mixed population. The inoculum is streaked over the agar surface in such a way that it "thins out" the bacteria. Some individual bacterial cells are separated and well-spaced from each other. As the original sample is diluted by streaking it over successive quadrants, the number of organisms decreases. Usually, by the third or fourth quadrant, only a few organisms are transferred which will give discrete colony forming units (CFUs)

Principle of Streaking

The sample/inoculum is diluted by streaking it across the surface of the agar plate. While streaking in successive areas of the plate, the inoculum is diluted to the point where there is only one bacterial cell deposited every few millimeters on the surface of the agar plate. When these lone bacterial cells divide and give rise to thousands and thousands of new bacterial cells, an isolated colony is formed. Pure cultures can be obtained by picking well-isolated colonies and re-streaking these on fresh agar plates.

A common assumption is an isolated colony of bacteria is the progeny of a single bacterial cell (i.e. colony is the clone). However, this is not necessarily true. With species in which the cells form a characteristic grouping during cell divisions, the colony-forming unit may develop from a group of cells rather than form a single cell. For example, clusters of staphylococci, chains of streptococci, etc**.**

Materials required

A source of bacteria (stock culture, previously streaked agar plate or any other inoculum)

Inoculation loop

A striker/lighter

Bunsen burner

Lysol (10%v/v)

Agar plate (nutrient agar or any other agar medium)

Paper towels

Tips for the best results:

Use only a small amount of inoculum.

Streak lightly so that you do not gouge the agar.

Flame the loop after you streak each quadrant.

Make sure the surface of the plate is free of droplets of condensed moisture.

Purpose of streaking

To produce isolated colonies of an organism (mostly bacteria) on an agar plate. This is useful when we need to separate organisms in a mixed culture (to purify/isolate particular strain from contaminants) or when we need to study the colony morphology of an organism.

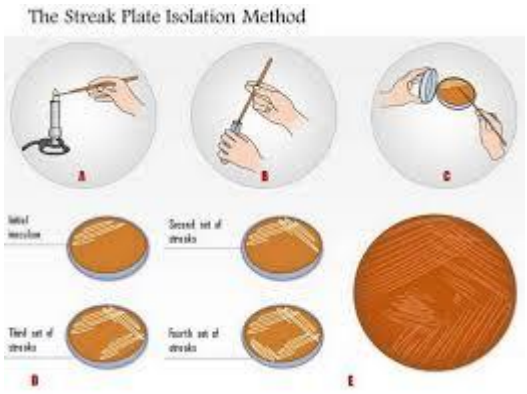
To identify the organism: biochemical tests to identify bacteria are only valid when performed on pure cultures.

Procedure

Sterilize the inoculating loop in the bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool. Pick an isolated colony from the agar plate culture and spread it over the first quadrant (approximately 1/4 of the plate) using close parallel streaks or Insert your loop into the tube/culture bottle and remove some inoculum. Immediately streak the inoculating loop very gently over a quarter of the plate using a back and forth motion . Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2). Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3). Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate (area 4). Flame your loop once more**.**

Results

The streaked plate is incubated at 37°C for 24 hours. Examine the colonies grown in the plate carefully. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate to obtain a pure culture.



Bacterial Growth

Bacterial growth is proliferation of bacterium into two daughter cells, in a process called binary fission. Providing no event occurs, the resulting daughter cells are genetically identical to the original cell. Hence, bacterial growth occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry, direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements**.**

Bacterial growth curve

In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: lag phase (A), log phase or exponential phase (B), stationary phase (C), and death phase (D).

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

The log phase (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day and then they can triple their population. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a “smooth,” horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase. Bridges et al. (2001) presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.

At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria [8]) and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as continuous culture. It is ideally spatially unstructured and temporally unstructured, in a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats. When Escherichia coli is growing very slowly with a doubling time of 16 hours in a chemostat most cells have a single chromosome.

Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria. In a synecological, true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual. Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms or agar surfaces present additional complex growth models.

Environmental conditions

Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels, and toxins. Conditions tend to be relatively consistent between bacteria with the exception of extremophiles. Bacterium have optimal growth conditions under which they thrive, but once outside of those conditions the stress can result in either reduced or stalled growth, dormancy (such as formation spores), or death. Maintaining sub-optimal growth conditions is a key principle to food preservation.

Temperature

Low temperatures tend to reduce growth rates which has led to refrigeration being instrumental in food preservation. Depending on temperature, bacteria can be classified as:-

Psychrophiles

Psychrophiles are extremophilic cold-loving bacteria or archaea with an optimal temperature for growth at about 15 °C or lower (maximal temperature for growth at 20 °C, minimal temperature for growth at 0 °C or lower). Psychrophiles are typically found in Earth's extremely cold ecosystems, such as polar ice-cap regions, permafrost, polar surface, and deep oceans.

Mesophiles

Mesophiles are bacteria that thrive at moderate temperatures, growing best between 20° and 45 °C. These temperatures align with the natural body temperatures of humans, which is why many human pathogens are mesophiles.

Thermophiles

Survive under temperatures of 45° - 60 °C

Acidity

Optimal acidity for bacteria tends to be around pH 6.5 to 7.0 with the exception of acidophiles. Some bacteria can change the pH such as by excreting acid resulting in sub-optimal conditions.

Water activity

Oxygen

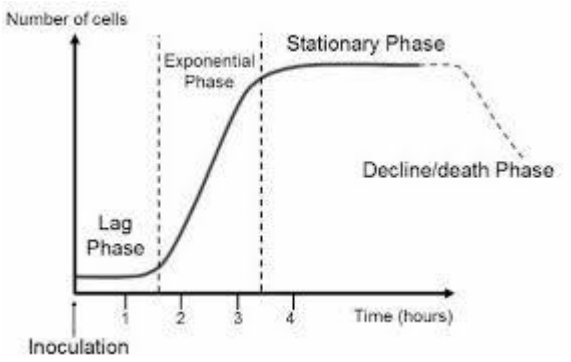
Bacteria can be aerobes or anaerobes. depending on the degree of oxygen required bacteria can fall into the following classes;

1.facultative-anaerobes-ie aerotolerant absence or minimal oxygen required for their growth

2.obligate-anaerobes grow only in complete absence of oxygen

3.facultative aerobes-can grow either in presence or minimal oxygen

4.obligate aerobes-grow only in the presence of oxygen



Factor affecting bacterial growth

Growth of bacteria is affected by many factors such as nutrition concentration and other environmental factors.

Some of the important factors affecting bacterial growth are:

Nutrition concentration

Temperature

Gaseous concentration

pH

Ions and salt concentration

Available water

1. Nutrient concentration: If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.

Different bacteria have different nutritional requirement.

The relationship between substrate concentration (nutrition) and growth rate

With increase in concentration nutrition, growth rate of bacteria increases up to certain level and then growth rate remains constant irrespective of nutrition addition.

2. Temperature:

Temperature affects the growth of bacteria by various ways.

The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.

There is no growth below minimum and above maximum temperature.

Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.

Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.

When temperature is increases continuously from its minimum, growth rate of bacteria increases because the rate of metabolic reaction increases with increase in temperature.

At certain temperature the growth rate become maximum, this temperature is known as optimal temperature.

On further increasing the temperature above optimal, growth rate decreases abruptly and completely ceases with reaching maximum temperature.

3. pH:

pH affects the ionic properties of bacterial cell so it affects the growth of bacteria.

Most of the bacteria grow at neutral pH (60.5-7.5). However there are certain bacteria that grow best at acidic or basic pH.

4. Ions and salt:

All bacteria requires metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++,Mn++ etc to synthesize enzymes and proteins.

Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt.

There is some halophilic bacteria such as Archeobacteria that require high concentration of salt in media.

5. Gaseous requirement:

Oxygen and carbon-dioxide are important gases that affects the growth of bacteria.

Oxygen is required for aerobic respiration and obligate aerobic bacteria must require O2 for growth. Eg. Mycobacterium, Bacillus

For obligate anaerobes Oxygen is harmful or sometime lethal. However facultative anaerobes can tolerate low concentration of O2.

Carbon-dioxide is needed for capnophilic bacteria. Such as Campylobacter, Helicobacter pylori

6. Available water:

Water is the most essential factor for bacterial growth.

Available water in the culture media determines the rate of metabolic and physiological activities of bacteria.

Sugar, salts and other substances are dissolved in water and are made available for bacteria.

Bacterial recombination

Bacterial recombination is a type of genetic recombination in bacteria characterized by DNA transfer from one organism called donor to another organism as recipient. This process occurs in three main ways

Transformation, the uptake of exogenous DNA from the surrounding environment.

Transduction, the virus-mediated transfer of DNA between bacteria.

Conjugation, the transfer of DNA from one bacterium to another via cell-to-cell contact.

The final result of conjugation, transduction, and/or transformation is the production of genetic recombinants, individuals that carry not only the genes they inherited from their parent cells but also the genes introduced to their genomes by conjugation, transduction, and/or transformation.

Recombination in bacteria is ordinarily catalyzed by a RecA type of recombinase. These recombinases promote repair of DNA damages by homologous recombination.

The ability to undergo natural transformation is present in at least 67 bacterial species. Natural transformation is common among pathogenic bacterial species. In some cases, the DNA repair capability provided by recombination during transformation facilitates survival of the infecting bacterial pathogen. Bacterial transformation is carried out by numerous interacting bacterial gene products.

Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through a pilus. It is a parasexual mode of reproduction in bacteria.

It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

Classical E. coli bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs, and indeed no generation of a new organism: instead an existing organism is transformed. During classical E. coli conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the recipient cell does not already contain a similar element.

The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. Others elements can be detrimental and may be viewed as bacterial parasites. Conjugation in Escherichia coli by spontaneous zygogenesis] and in Mycobacterium smegmatis by distributive conjugal transfer differ from the more well studied classical E. coli conjugation in that these cases involve substantial blending of the parental genomes.

Donor cell produces pilus. Pilus attaches to recipient cell and brings the two cells together.

The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.

Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor.

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the oriV, and an origin of transfer, or oriT. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called F-positive or F-plus (denoted F+). Cells that lack F plasmids are called F-negative or F-minus (F−) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a tra and trb locus, which together are about 33 kb long and consist of about 40 genes. The tra locus includes the pilin gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F− bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the tra or trb locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the relaxase enzyme creates a nick in one of the strands of the conjugative plasmid at the oriT. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a relaxosome. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or T-strand, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the oriV) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

1.The insertion sequences (yellow) on both the F factor plasmid and the chromosome have similar sequences, allowing the F factor to insert itself into the genome of the cell. This is called homologous recombination and creates an Hfr (high frequency of recombination) cell. 2.The Hfr cell forms a pilus and attaches to a recipient F- cell. 3.A nick in one strand of the Hfr cell's chromosome is created. 4.DNA begins to be transferred from the Hfr cell to the recipient cell while the second strand of its chromosome is being replicated. 5.The pilus detaches from the recipient cell and retracts. The Hfr cell ideally wants to transfer its entire genome to the recipient cell. However, due to its large size and inability to keep in contact with the recipient cell, it is not able to do so. 6.a. The F- cell remains F- because the entire F factor sequence was not received. Since no homologous recombination occurred, the DNA that was transferred is degraded by enzymes. b. In very rare cases, the F factor will be completely transferred and the F- cell will become an Hfr cell.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain ["High Frequency of Recombination"]) some of the donor's chromosomal DNA may also be transferred with the plasmid DNA The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common

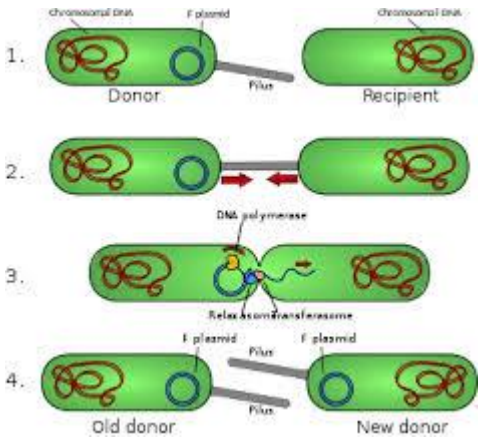
laboratory strains of E. coli the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called Hfr (high frequency of recombination). The E. coli genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.

In addition to classical bacterial conjugation described above for E. coli, a form of conjugation referred to as spontaneous zygogenesis (Z-mating for short) is observed in certain strains of E. coli. In Z-mating there is complete genetic mixing, and unstable diploids are formed that throw off phenotypically haploid cells, of which some show a parental phenotype and some are true recombinants.

Genetic engineering applications

Conjugation is a convenient means for transferring genetic material to a variety of targets. In laboratories, successful transfers have been reported from bacteria to yeast, plants, mammalian cells, diatoms and isolated mammalian mitochondria. Conjugation has advantages over other forms of genetic transfer including minimal disruption of the target's cellular envelope and the ability to transfer relatively large amounts of genetic material (see the above discussion of E. coli chromosome transfer). In plant engineering, Agrobacterium-like conjugation complements other standard vehicles such as tobacco mosaic virus (TMV). While TMV is capable of infecting many plant families these are primarily herbaceous dicots. Agrobacterium-like conjugation is also primarily used for dicots, but monocot recipients are not uncommon.



Transformation

In molecular biology and genetics, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacterium must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from one bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium). In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

History

Transformation in bacteria was first demonstrated in 1928 by the British bacteriologist Frederick Griffith. Griffith was interested in determining whether injections of heat-killed bacteria could be used to vaccinate mice against pneumonia. However, he discovered that a non-virulent strain of Streptococcus pneumoniae could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of S. pneumoniae and using just this DNA were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation" (See Avery-MacLeod-McCarty experiment) The results of Avery et al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other methods of genetic transfer (conjugation in 1947 and transduction in 1953) by Joshua Lederberg that Avery's experiments were accepted.

It was originally thought that Escherichia coli, a commonly used laboratory organism, was refractory to transformation. However, in 1970, Morton Mandel and Akiko Higa showed that E. coli may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution. Two years later in 1972, Stanley Norman Cohen, Annie Chang and Leslie Hsu showed that $CaCl_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Douglas Hanahan. The discovery of artificially induced competence in E. coli created an efficient and convenient procedure for transforming bacteria which allows for simpler molecular cloning methods in biotechnology and research, and it is now a routinely used laboratory procedure. Transformation using electroporation was developed in the late 1980s, increasing the efficiency of in-vitro transformation and increasing the number of bacterial strains that could be transformed.Transformation of animal and plant cells

was also investigated with the first transgenic mouse being created by injecting a gene for a rat growth hormone into a mouse embryo in 1982.In 1907 a bacterium that caused plant tumors, Agrobacterium tumefaciens, was discovered and in the early 1970s the tumor-inducing agent was found to be a DNA plasmid called the Ti plasmid. By removing the genes in the plasmid that caused the tumor and adding in novel genes, researchers were able to infect plants with A. tumefaciens and let the bacteria insert their chosen DNA into the genomes of the plants. Not all plant cells are susceptible to infection by A. tumefaciens, so other methods were developed, including electroporation and micro-injection. Particle bombardment was made possible with the invention of the Biolistic Particle Delivery System (gene gun) by John Sanford in the 1980s.

Transformation is one of three forms of horizontal gene transfer that occur in nature among bacteria, in which DNA encoding for a trait passes from one bacterium to another and is integrated into the recipient genome by homologous recombination; the other two are transduction, carried out by means of a bacteriophage, and conjugation, in which a gene is passed through direct contact between bacteria. In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

Competence refers to a temporary state of being able to take up exogenous DNA from the environment; it may be induced in a laboratory.

It appears to be an ancient process inherited from a common prokaryotic ancestor that is a beneficial adaptation for promoting recombinational repair of DNA damage, especially damage acquired under stressful conditions. Natural genetic transformation appears to be an adaptation for repair of DNA damage that also generates genetic diversity.

Transformation has been studied in medically important Gram-negative bacteria species such as Helicobacter pylori, Legionella pneumophila, Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae and Vibrio cholerae. It has also been studied in Gram-negative species found in soil such as Pseudomonas stutzeri, Acinetobacter baylyi, and Gram-negative plant pathogens such as Ralstonia solanacearum and Xylella fastidiosa. Transformation among Gram-positive bacteria has been studied in medically important species such as Streptococcus pneumoniae, Streptococcus mutans, Staphylococcus aureus and Streptococcus sanguinis and in Gram-positive soil bacterium Bacillus subtilis. It has also been reported in at least 30 species of Proteobacteria distributed in the classes alpha, beta, gamma and epsilon. The best studied Proteobacteria with respect to transformation are the medically important human pathogens Neisseria gonorrhoeae , Haemophilus influenzae and Helicobacter pylori

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

Natural competence and transformation

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

Naturally competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s). The transport of the exogenous DNA into the cells may require proteins

that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

Due to the differences in structure of the cell envelope between Gram-positive and Gram-negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells, however most of them share common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase. Only single-stranded DNA may pass through, the other strand being degraded by nucleases in the process. The translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process. In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane. Pilin may be required for competence, but its role is uncertain. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake**.**

Competence for transformation is typically induced by high cell density and/or nutritional limitation, conditions associated with the stationary phase of bacterial growth. Transformation in Haemophilus influenzae occurs most efficiently at the end of exponential growth as bacterial growth approaches stationary phase. Transformation in Streptococcus mutans, as well as in many other streptococci, occurs at high cell density and is associated with biofilm formation. Competence in B. subtilis is induced toward the end of logarithmic growth, especially under conditions of amino acid limitation. Similarly, in Micrococcus luteus (a representative of the less well studied Actinobacteria phylum), competence develops during the mid-late exponential growth phase and is also triggered by amino acids starvation.

By releasing intact host and plasmid DNA, certain bacteriophages are thought to contribute to transformation.

Transformation, as an adaptation for DNA repair

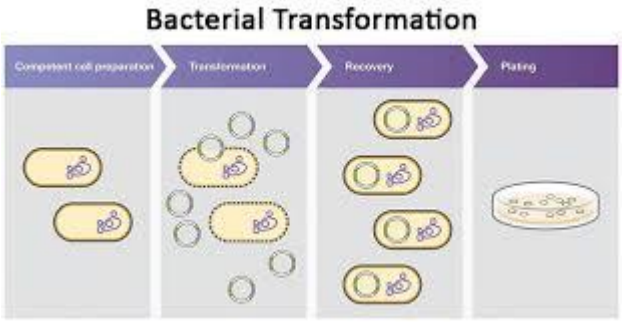
Competence is specifically induced by DNA damaging conditions. For instance, transformation is induced in Streptococcus pneumoniae by the DNA damaging agents mitomycin C (a DNA cross-linking agent) and fluoroquinolone (a topoisomerase inhibitor that causes double-strand breaks).In B. subtilis, transformation is increased by UV light, a DNA damaging agent. In Helicobacter pylori, ciprofloxacin, which interacts with DNA gyrase and introduces double-strand breaks, induces expression of competence genes, thus enhancing the frequency of transformation[36] Using Legionella pneumophila, Charpentier et al. tested 64 toxic molecules to determine which of these induce competence. Of these, only six, all DNA damaging agents, caused strong induction. These DNA damaging agents were mitomycin C (which causes DNA inter-strand crosslinks), norfloxacin, ofloxacin and nalidixic acid (inhibitors of DNA gyrase that cause double-strand breaks[38]), bicyclomycin (causes single- and double-strand breaks[39]), and hydroxyurea (induces DNA base oxidation[40]). UV light also induced competence in L. pneumophila. Charpentier et al. suggested that competence for transformation probably evolved as a DNA damage response.

Logarithmically growing bacteria differ from stationary phase bacteria with respect to the number of genome copies present in the cell, and this has implications for the capability to carry out an important DNA repair process. During logarithmic growth, two or more copies of any particular region of the chromosome may be present in a bacterial cell, as cell division is not precisely matched with chromosome replication. The process of homologous recombinational repair (HRR) is a key DNA repair process that is especially effective for repairing double-strand damages, such as double-strand

breaks. This process depends on a second homologous chromosome in addition to the damaged chromosome. During logarithmic growth, a DNA damage in one chromosome may be repaired by HRR using sequence information from the other homologous chromosome. Once cells approach stationary phase, however, they typically have just one copy of the chromosome, and HRR requires input of homologous template from outside the cell by transformation.

It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure, making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance across the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

Electroporation is another method of promoting competence. In this method the cells are briefly shocked with an electric field of 10-20 kV/cm, which is thought to create holes in the cell membrane through which the plasmid DNA may enter. After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.



Transduction

Transduction is a mode of genetic transfer from one bacteria to another through a virus. There is no direct contact between the bacterial cells. The other ways of genetic recombination in bacteria include transformation and conjugation. In this process, bacteriophages, which infect bacteria, use host cells to multiplicate and while assembling they sometimes pack the bacterial DNA with them. Later, when these viruses infect new bacterial cells, the bacterial genome that they carry may get inserted into the host genome.

Transduction is commonly used in genetic engineering for inserting foreign DNA into the host cell. Transduction was discovered by Zinder and Lederberg in Salmonella. Hershey and Chase used transduction as a tool to confirm that DNA is the genetic material.

In transduction, the transfer of bacterial DNA depends on viral infection. The steps involve:

Infection of the bacterial cell by bacteriophage. The virus uses the host machinery to make multiple copies either directly by the lytic cycle or first gets incorporated into the bacterial genome by the lysogenic cycle and followed by the lytic stage.

During assembly of bacteriophages, the bacterial genome also gets packed by mistake in the viral head alongside the viral genome. In the lysogenic cycle, during excision of prophage, some parts of the bacterial genome that flank the prophage are also excised and go inside the assembled viral head together with the viral genome. When these viruses infect another bacterial cell, they inject the viral DNA as well as donor DNA into the host cell.

The bacterial DNA either forms plasmids or gets inserted into the recipient DNA if it is homologous to the recipient genome. Most of the time it remains as an extrachromosomal DNA. It can also get inserted with the prophage if it is a temperate phage. So the fate depends on the portion of bacterial DNA and also on the nature of bacteriophages.

Types of Transduction

Transduction is common in both virulent and temperate phages, i.e. by lytic or lysogenic cycle. Transduction is of two types:

Generalized Transduction – In this, the phage can carry any part of DNA.

Specialized Transduction – In this, the phage carries only the specific part of DNA.

Generalized Transduction

Generalized transduction can occur by both lytic or lysogenic cycle. Here, any random part of DNA gets packed in bacteriophages by mistake along with the viral genome. It occurs at the lytic stage of the phage life cycle.

When the virus-containing bacterial DNA infects another cell, it can get inserted into the host genome or if it was a plasmid, then it can reform the plasmid.

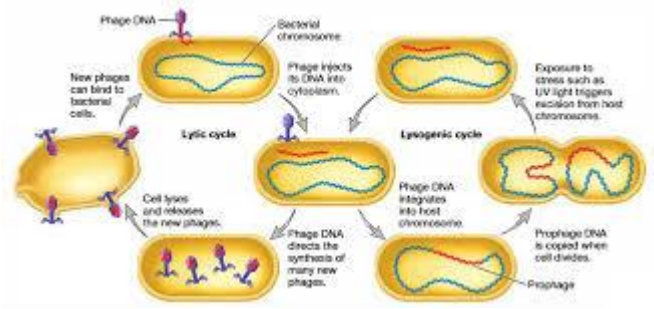
Generalized transduction is used to study linkage information, gene mapping, comparing genomes of two different bacteria, mutagenesis, etc. Example of generalized transduction includes E.coli transduction by P1 phage.

Specialized Transduction

Specialized transduction can occur only through the lysogenic cycle, i.e. by temperate phage. Here, only the specific part of the bacterial DNA is packed into the virus. It occurs when the prophage, i.e. viral DNA, which gets inserted into the bacterial genome in the lysogenic cycle excises. When prophage excises from bacterial DNA, some parts of bacterial DNA, which are flanked on both sides of the prophage are also excised. Here, the newly packed phage genome consists of both bacterial and viral genome. Later, when the virus with the recombinant genome infects a new bacterial cell, the bacterial gene also gets inserted into the host genome with the viral genome through lysogeny. The recipient cell now shows the newly acquired characteristics. Specialized transduction is commonly used for isolation and insertion of genes of choice. Example of specialized transduction includes E.coli transduction by λ phage.

Application of Transduction

Transduction is one of the most important tools for genetic engineering. Transduction is used to insert the genes of choices in animals and plant cells to modify the genetic constituents and get the desired characteristics. It can be used for gene therapy. It has huge potential to cure genetic diseases. It is an important tool in genetics and molecular biology research.


Phage DNA
Bacterial chromosome
Phage injects its DNA into cytoplasm.
New phages can bind to bacterial cells.
Lytic cycle
Lysogenic cycle
Exposure to stress such as UV light triggers excision from host chromosome.
Phage DNA integrates into host chromosome.
Cell lyses and releases the new phages.
Phage DNA directs the synthesis of many new phages.
Prophage DNA is copied when cell divides.
Prophage

Economic importance of Bacteria

A. Beneficial Activities of Bacteria:

There are many kinds of bacteria without which we could not live. They are absolutely essential to the presence of life on earth. They make possible the continued existence of green plants and therefore of animals because the plants are the only source of food for animals.

Following is a brief account of the more important activities of the bacteria:

1. Role in Agriculture:

(i) Decay and decomposition:

Soil bacteria play an important role in brining about decomposition of organic matter. They serve a double purpose. In the first instance they act as scavengers removing harmful waste from the earth. Secondly, they return it to the soil as plant food. The dead bodies and wastes of organisms (both plants and animals) are decomposed by the activities of the saprophytic bacteria. In consequence a variety of elements of minerals of the earth such as carbon, oxygen, hydrogen, sulphur and phosphorus which make up their bodies are reduced to simple compounds such as carbon monoxide, water, nitrates, sulphates and phosphates. Some of these go back to the soil and the rest to the air. From the soil they can be absorbed as plant food. This activity of the bacteria is used in sewage disposal system of cities. The bacterial action on the city's sewage promotes decay. As a result water is finally purified and changed into an odourless and valuable fertiliser instead of a dangerous and expensive waste product.

(ii) Soil fertility:

Some bacteria play an important role in maintaining and others in increasing soil fertility. The fertility of soil is proportional to its nitrogen content. Nitrogen is an essential ingredient of all living protoplasm. All growing plants, therefore, require it in their metabolism. Atmosphere, no doubt, is four-fifths (80%) nitrogen, green plants generally are unable to use it. They mostly absorb it as nitrates and to some extent as ammonia from the soil. Continuous absorption of these salts results in their exhaustion in the soil. Nearly all fertilisers for the soil include a large proportion of such soluble nitrogen compounds to promote plant growth.

In nature the presence of a regular supply of these salts is ensured by bacteria of certain types. These bacteria which function as Nature's farmers belong to three categories, namely, ammonifying bacteria, nitrifying bacteria and nitrogen-fixing bacteria. They are the agents of maintaining a continual circulation of nitrogen in nature between the plant world, in soil and the atmosphere. The series of changes through which the nitrogen passes due to the activities of these organisms constitute the nitrogen cycle .

Nitrogen fixing bacteria

(a) Ammonifying Bacteria:

The saprophytic bacteria break down the proteins and other nitrogen containing remains of the plant and animal origin in the soil to amino acids by secreting enzymes.

The amino acids are then converted into ammonia by a group of bacteria called the ammonifying bacteria. The liberated ammonia may combine with carbon dioxide and water in the soil to form

ammonium carbonate. A few plants such as the common cereals can make use of ammonium compounds as a source of nitrogen. The majority of plants, however, cannot absorb ammonium compounds as a source of nitrogen.

(b) Nitrifying Bacteria:

Ammonia is very soluble. It moves in the soil rapidly and is acted upon by microorganisms of the category of chemosynthetic autotrophs in the soil. They are the nitrifying bacteria such as Nitrosomonas and Nitmbacter. They form nitrates from ammonium compounds.

The reaction takes place in the following two steps:

Nitrifying Bacteria

Nitrosomonas oxidizes ammonium carbonate to nitrous acid liberating energy. The nitrous acid then combines with bases in the soil forming potassium nitrite. Nitrobacter oxidizes nitrites to nitrates again liberating energy.Neither the ammonifying nor the nitrifying bacteria add to the total quantity of combined nitrogen in the soil. The ammonifying bacteria convert amino acids into ammonia. The process is called ammonification. The nitrifying bacteria convert nitrogen from the unavailable form of ammonium salts to the available nitrates. This process converting unavailable ammonium salts into available nitrates is called nitrification.

(c) Nitrogen-fixing Bacteria:

A considerable amount of nitrogen is lost by denitrification and through drainage. The loss must be made good by equal gains if the soil fertility is to be maintained. The electric discharges in the atmosphere bring about the formation of traces of nitrogen compounds which are washed to the soil by rain water. The largest additions, however, come from a biological fixation process through the activity of two types of nitrogen-fixing bacteria. Some of them live free in the soil and others in the root nodules of leguminous plants. They are able to make use of the atmospheric nitrogen and change it into nitrogenous compounds. The nitrogen-fixing bacteria are thus unique in tapping a source of nitrogen not available to most other plants. This process of nitrogen transformation is called nitrogen fixation.

Examples - Fixing Bacteria

(i) Azotohacter heijerinckia (aerobic forms) and Clostridium (anaerobic) live free in the soil:

They take gaseous nitrogen from the air present between the soil particles. The nitrogen combines with other elements forming organic nitrogenous compounds. These compounds are assimilated by the bacteria. In due course these bacteria die and their dead bodies containing nitrogenous compounds are decomposed by another type of bacteria called the bacteria of decomposition. During decomposition ammonia is produced. The nitrifying bacteria convert this ammonia first into nitrites and finally into nitrates. Nitrates constitute the form of nitrogen needed by the green plants.

(ii) Rhizobium leguminosarum (syn. Bacillus radicicola) is another nitrogen-fixing bacterium:

It lives in the roots of such plants as Pea (Fig. 18.19A), Bean, Medicago and others. All these belong to the Pea family (Leguminoseae). Besides the legumes, the nodules are found on the roots of Alnus glutinosa, Casuarina, species of Coriaria and a few others.

The symbiont in non- leguminous plants is a member of Plasmodiophorales. The presence of bacteria in the roots causes the formation of little nodules (Fig. 18.19A). In Pavetta indica the bacterial nodules are formed on the leaves.

Rhizobium Leguminosarum -These nodules or the tubercles are the homes of millions of these bacteria. They have the ability to take up free nitrogen of the air and convert it into nitrogen compounds. A part of the fixed nitrogen passes into the tissues of legume plant and a part diffuses into the surrounding soil. These bacteria enter into a mutually beneficial partnership with the host plant. They give to the host the nitrogen compounds and receive in return carbohydrates manufactured by the host plant. This association is an excellent example of symbiosis. The legumes are very rich in nitrogen because of this association.

Some of our best protein plant foods come from the legume family of plants. The legume plants can flourish on land that has been depleted of its nitrogen by other plants. They are sometimes grown and ploughed under by the farmer when a foot or so in height.

The decaying bodies of these legume plants enrich the soil. They furnish a rich supply of nitrogen to the future crop. This is called green manuring. The soil on which repeated crops of cereals are grown becomes impoverished. It can be enriched again by growing on it a crop of some plants of Pea family. This practice of alternating cereals with leguminous crop is known as rotation of crops. The leguminous plants contain more of nitrogen than they get from the soil salts. The additional quantity is obtained from the air by Rhizobium. Neither Rhizobium nor the legume root alone can fix nitrogen.

2. Role of Bacteria in Industries:

Man has utilised the activities of bacteria for various industrial processes. The butter and cheese industries entirely depend upon the activities of the lactic acid bacteria.

The souring and curding of milk by lactic acid bacteria is another common example of application in everyday life. It takes place in two steps. In the first step the lactose sugar of milk is fermented into glucose by an enzyme lactose secreted by the lactic acid bacteria.

In the second step there is transformation of glucose into lactic acid. The latter sours the milk and coagulates the milk proteins (casein) forming curds and whey. Oxidation of alcohol into vinegar (acetic acid) is brought about by the acetic acid bacteria.

The curing of tea, tobacco and manufacture of indigo are other examples of useful chemical activities of bacteria which have been controlled for the benefit of mankind. The process of tanning hides in leather making and preparing sponges also involve the use of bacteria.

The production of linen is impossible without bacterial activity. The tough fibres, which are left behind, are separated. These fibres are spun and woven into linen cloth, ropes, etc.

The preparation of coffee and cocoa is also dependent upon bacterial action. The cocoa beans are white in colour and quite bitter in taste. The bacteria digest the bitter coverings of seeds and give the characteristic colour, flavour and aroma.

Many saprophytic bacteria in their metabolic activities excrete waste products of great commercial importance.

Samples Produced from Butter and Cheese Industry

Preparing Sponges

Some of these are:

(i) Lactic acid: It is useful in tanning industries.

(ii) Citric acid: It is used to give aroma and flavour to beverages, sweets and other foodstuffs.

(iii) Vitamins: Vitamin B is the product of fermentation of sugars and starch by Clostridium acetobutilicum. The vitamins are used in medicinal preparations.

(iv) Butyl alcohol: Butyl alcohol, acetone and ethyl alcohol are produced in one fermentation operation when a certain bacterium is allowed to act on cooked com starch. These products are important commercial solvents.

(v) Acetone: It is an important ingredient of explosives and is also used in the manufacture of photographic films.

3. Role of Bacteria in Medicine:

1. Source of Antibiotics:

The milder antibiotics of bacterial origin are tyrothricin, subtilin, polymyxin B, and bacitracin. Bacillius subtilis is the source of subtilin. Bacitracin is obtained from a stain very much like B. subtilis. The actionomycetes which are filamentous, bacteria-like organisms produce more powerful antibiotics such as streptomycin, aureomycin and terramycin.

2. Preparation of Serums and Vaccines:

These are substances which are used to develop immunity to various diseases in man. Serums are used in advance as a therapeutic measure. They are also used when a person actually suffers from a disease. Diphtheria, lockjaw, pneumonia, etc. are the diseases in which the serums are effective. Vaccines are commonly used to make people immune to diseases like typhoid, small-pox, cholera, scarlet fever, etc. In the preparation of serums, small doses of bacterial toxins (poisons) are injected into the blood of animals such as horses. To combat or neutralize the bacterial poisons, the body of the animal produces antibodies. The blood of the animal is then withdrawn. Impurities such as blood corpuscles and other solid matter are removed from the blood. The clear blood liquid containing the antibodies is the serum. It is used as weapon to combat diseases caused by these bacteria.

To produce vaccines dead or weakened disease producing bacteria or their diluted poisons (antigens) are directly injected into a man to cause a disease in a mild form. As a reaction the host is stimulated to form antibodies. The latter may remain for years in the body of the host imparting immunity against the same type of bacteria which may later enter his body.

B. Harmful Activities of Bacteria:

1. Food Poisoning:

Of course, all activities of bacteria are not beneficial. Some saprophytic bacteria cause decay of our food and make it unpalatable. The activities of certain bacteria produce powerful toxins such as ptomains in the food. These toxins are powerful enough to cause food poisoning which results in serious illness and even death. Some species of Staphylococcus are the common offenders.

There is another dangerous food poisoning bacterium known as Clostridium botulinium. It causes botulism—a fatal form of food-poisoning.

2. Disease:

Many parasitic bacteria are the causative agents of bacterial diseases. They cause diseases of our economic plants, domesticated animals and man. T.J. Burrill in 1878 first gave the information that bacteria cause plant diseases. There are more than 170 species of bacteria which cause plant diseases. Usually they are rod-like and non-spore forming. Many of them have flagella. The bacteria gain entry into the host through wounds or natural openings such as stomata, lenticels, hydathodes or through the thin epidermis.

The bacterial diseases of plants belong to the following categories:

(i) Wilt diseases caused by blocking of the vessels of host plant by masses of bacteria. The common example of this category are the wilt diseases of potato, cucumber, water melon and eggplant.

(ii) Crown gall and Hairy root diseases. These are due to overgrowth or hyperplasia. The crown gall of beets and hairy root of apple are the examples.

(iii) Narcotic blights, leaf spots and rots caused by killing of parenchyma cells. Fire blight of apple, and pear and soft rot of carrot and turnip are the common examples. The following Table 3 gives a list of some important disease-causing bacteria, host plants and diseases.

3. Denitrification:

There are, sometimes, a group of bacteria in the soil which reverse the nitrifying process. They injure the soil by causing the loss of a part of its combined nitrogen.

This they do by breaking down nitrates into nitrites and nitrites into ammonia compounds or to free nitrogen.

$NO_3 \rightarrow NO_2 \rightarrow NH_3 \rightarrow$ N gas $\uparrow$

The free nitrogen passes into the atmosphere and is lost to the soil. The result is the lowering of soil fertility. This process is called denitrification. The bacteria which bring about denitrification are called the denitrifying bacteria. They are most active in the soil containing excess of nitrogen compounds such as the heavily manured soils. Soils deficient in oxygen are also favourable for the activity of this type of bacteria.

Denitrification is checked if the soil is well aerated by ploughing or digging and well drained. It is uneconomic to use natural and artificial nitrate manures simultaneously. The denitrifying bacteria found in the faeces contained in the manures tend to destroy the nitrates.