INTRODUCTION TO MICROBIOLOGY AND MEDICINE

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Microbiology

is the study of microorganisms which are organisms so small that they can not be seen with the naked eye.,

They can be observed only with the aid of a microscope.

Microorganisms are found almost everywhere in nature. They are in constant interaction with human and other life cycles. Some bacteria, for example, are normal constituents of the human intestine

they can cause diseases. These microorganisms are known as pathogens. Those organisms that do not cause any harm are called commensals.

Taxonomy and classification of Microorganisms

Before the discovery of microorganisms, all living things were thought to belong to either the animal or the plant kingdom. In 1675, Anthony von Leeuwenhoek, a Dutch draper, described little animals (animalcules) which he saw with the aid of his sample home –made microscope while examining stagnant rain water. He described many kinds of cells which included protozoa, algae, yeasts and bacteria. He was the first on record to observe microorganisms. Many workers in different parts of the world did a lot of pioneering work in the study of microorganisms. But Louis Pasteur, a French chemist, was by far the most outstanding figure in this regard. As a result of his brilliant researches between 1860 and 1869, he was able to show that microorganisms caused diseases.

Classification based on evolutionary (ancestry) origins is called phylogenic classification, and that based on easily recognizable characteristics or features is called 'artificial' classification

Classification

In 1866, Haeckel proposed the establishment of a third and separate kingdom of living organisms, the Protista, which was defined to include algae, protozoa, fungi, viruses and bacteria. In 1957, Stanier divided the Protista into the eukaryotic (higher) and Prokaryotic (lower) groups based on cell types. The eukaryotic cells which are found in protozoa, fungi and algae, contain a nucleus bound by a nuclear membrane and undergo true mitosis. The prokaryotic organism possess a nuclear structure of a single deoxyribonucleic acid (DNA) ring with no nuclear membrane.

Table 1 Classification of the Protista

Higher Protista	Fungi Protozoa Algae	Eukaryotic cells possessing a nucleus bounded by a nuclear membrane which contains chromosomes and undergoes true mitosis
Lower Protista	Bacteria Blue – green algae	Prokaryotic cells possessing a nuclear structure which consists of a single ring or loop of deoxyribonucleic acid (DNA) concentrated in one part of the cell and considered to be a single chromosome

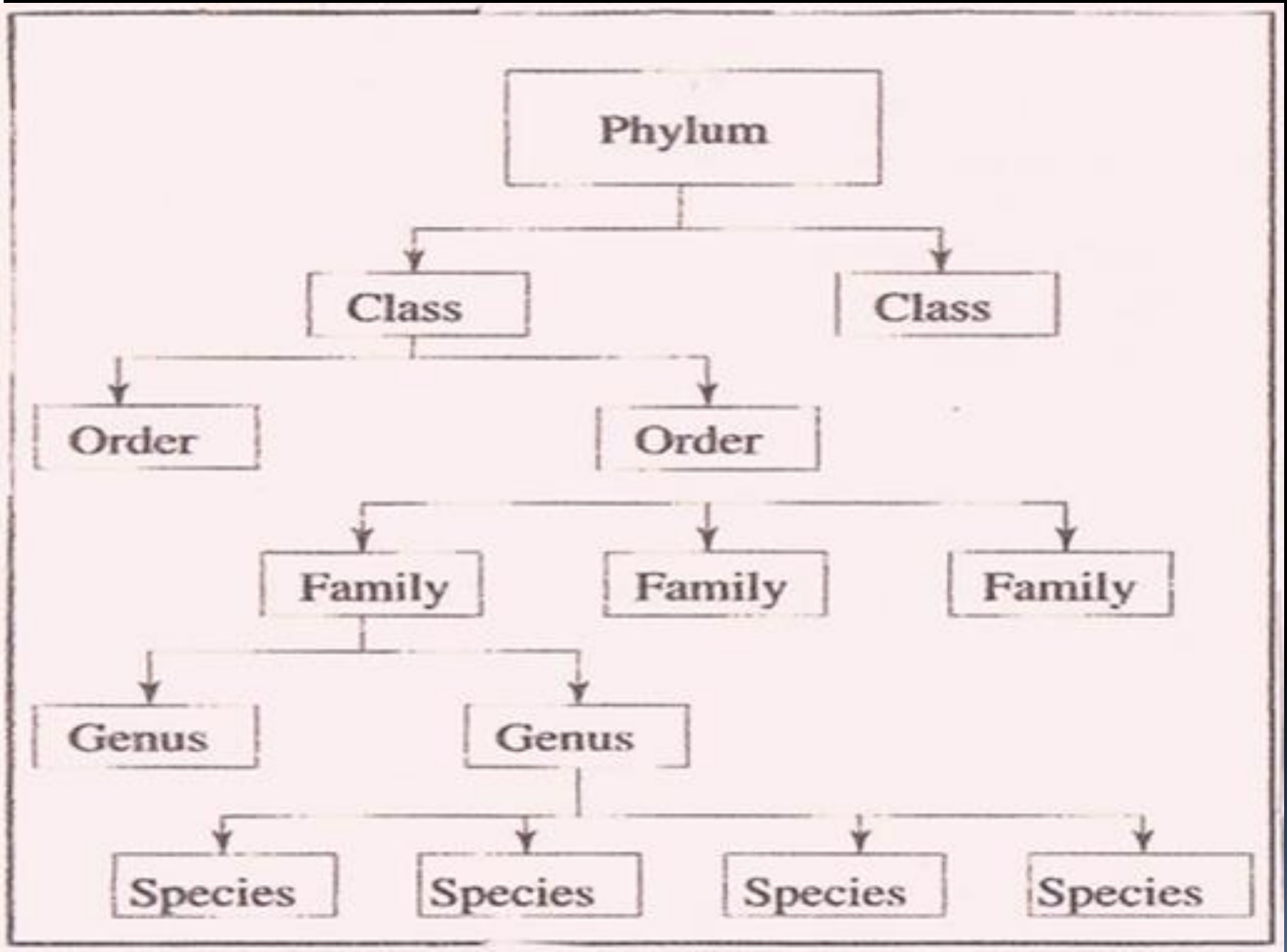

Phylum
Class
Class
Order
Order
Family
Family
Family
Genus
Genus
Species
Species
Species
Species

Table 2 Some important human pathogens from various groups of microorganisms and diseases caused by them bacteria

Organism	Disease
Staphylococcus aureus	Septicaemia, food poisoning, abscesses,carbuncles
Streptococcus pyogenes	Tonsilitis, erysipelas, scarlet fever
Streptococcus pneumoniae	Pneumonia, meningitis, septicaemia
Neisseria meningitidis	Meningitis, septicaemia
Neisseria gonorrhoeae	Gonorrhoea
Escherichia coli and other coliforms	Urinary tract infection, septicaemia, pyogenic infections
Salmonella typhi, paratyphi A, B and C	Enteric fever
Salmonella spp.	Food poisoning
Shigella spp.	Dysentry
Vibrio cholerae	Cholera
Proteus spp.	Urinary tract and wound infection
Haemophilus influenzae	Meningitis, pneumonia, epiglottitis, septicaemia
Bordetella paertusis	Whooping cough
Yersinia pestis	Plague
Brucella species	Undulant fever (Brucellosis)
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Corynebacterium diphtheriae	Diptheria
Clostridium perfringens	Gas gangrene
Clostridium tetani	Tetanus
Clostridium botulinum	Botulism
Bacillus anthracis	Anthrax
Legionella pneumophila	Legionaie's disease
Treponema pallidum	Syphilis
Chlamydia spp.	Trachoma, pneumonia, genital tract infection
Rickettsiae	Rickettsial pox, Q fever, Typhus fever

Morphology and Structure of Bacteria

The classification of bacteria in routine use is artificial and is based on some recognizable features and characteristics which have been arbitrarily selected. These feature include:

Morphology

Staining reaction

Cultural requirements

Biochemical reactions

Antigenic structure

DNA base composition i.e. guanine: Cytosine ratio

Morphology

Bacteria are microscopic unicellular organisms. The unit of measurement of bacteria is the micrometer (µm) and it is 1/1000 of a millimeter (0.001 µm). Morphological classification on bacteria is based on the following types of shapes of cells:

1. The spheroid or ovoid or coccus

These group of bacteria are popularly called cocci (plural of coccus). They measure about 0.5 -1 µm in diameter. Sometimes these cells are flattened or distorted to give a change in shape.

The bacteria multiply by binary fission. During multiplication, the daughter cell in attached to the parent cell but it gets detached before fission occurs again. The pairs of cocci seen are referred to as diploococci. If the fission continues while they remain attached, chains of cocci are formed, and are referred to as streptococci. If the division is not in one plain and clusters of cocci are formed randomly, they are known as staphylococci. Tetrads (or tetra cocci) are formed when the cocci remain in pairs for two consecutive division and form regular aggregates of four cocci. When the terms Staphylococcus and Streptococcus are used as generic names, the first letter is a capital letter.

2. Bacillus

This is a 'rod' shaped cell measuring about 1 -10 µm in length and 0.3 1.0µm in width.

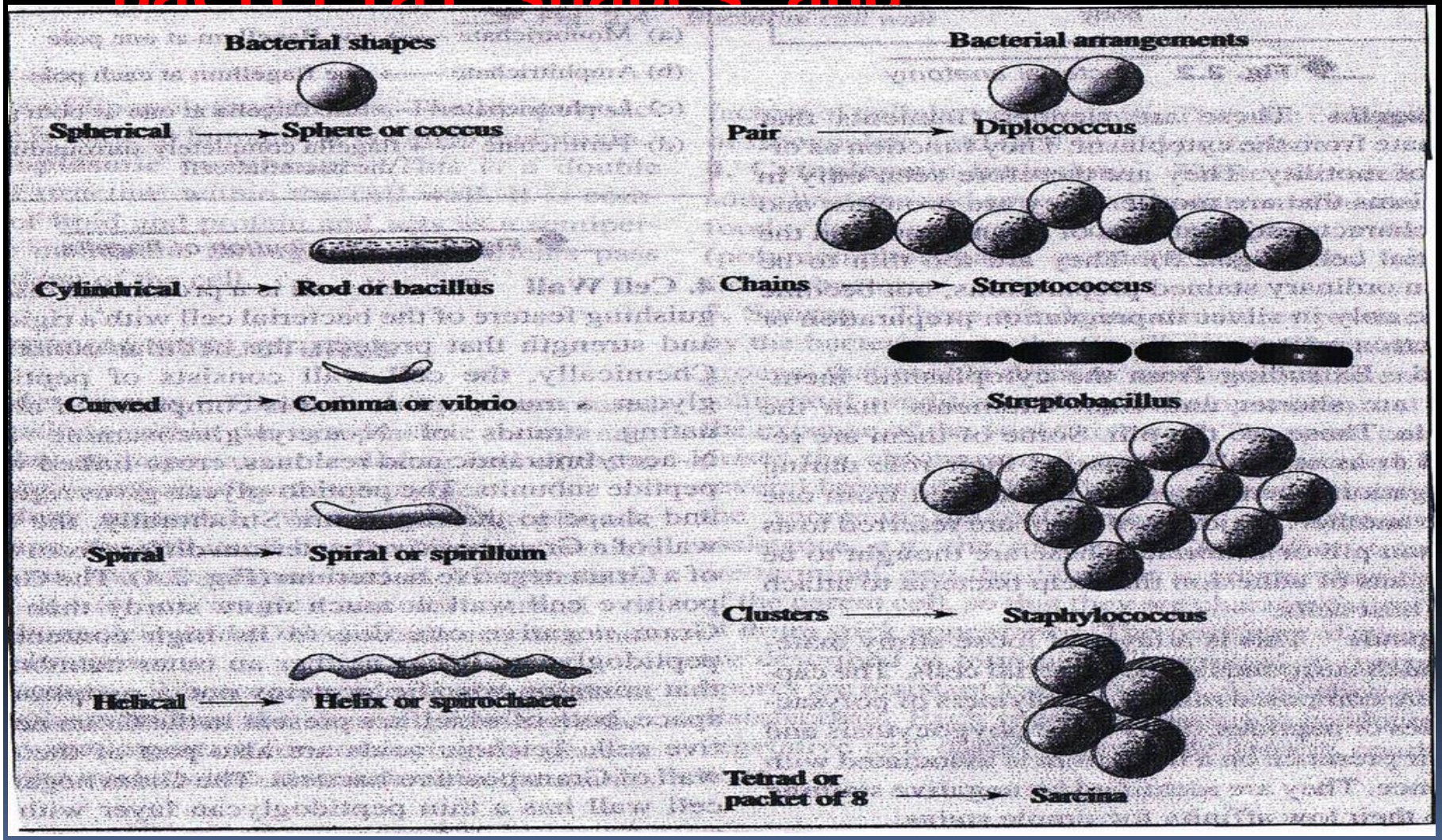
The bacilli (plural) or rods may remain attached after division and they are called sterptobacilli : or they may be arranged at varying angles to one another due to incomplete separation after cell division, thus giving the appearance of Chinese lettering. This is characteristic of the genus Corynebacterium.

The morphology of some of these bacilli may be affected as a result of the formation of spores during unfavorable conditions. Some species contain these spores in the centre with or without bulging and others may have them at terminal ends or towards one end.

5. Spirochaete

This is a flexible spiral shaped organism. It possesses an axial fibrearound which the body is twisted in a helical fashion. It measures about 10 – 20 µm in length and 0.2 -0.4 µm in thickness.

Figure B shows the various bacterial shapes and (6)



Bacterial Cell Structure
(Bacterial Anatomy)

External Structures

The external structure are those structures which protrude from the cell into the surrounding environment. They include:

Fig. C Bacterial Anatomy

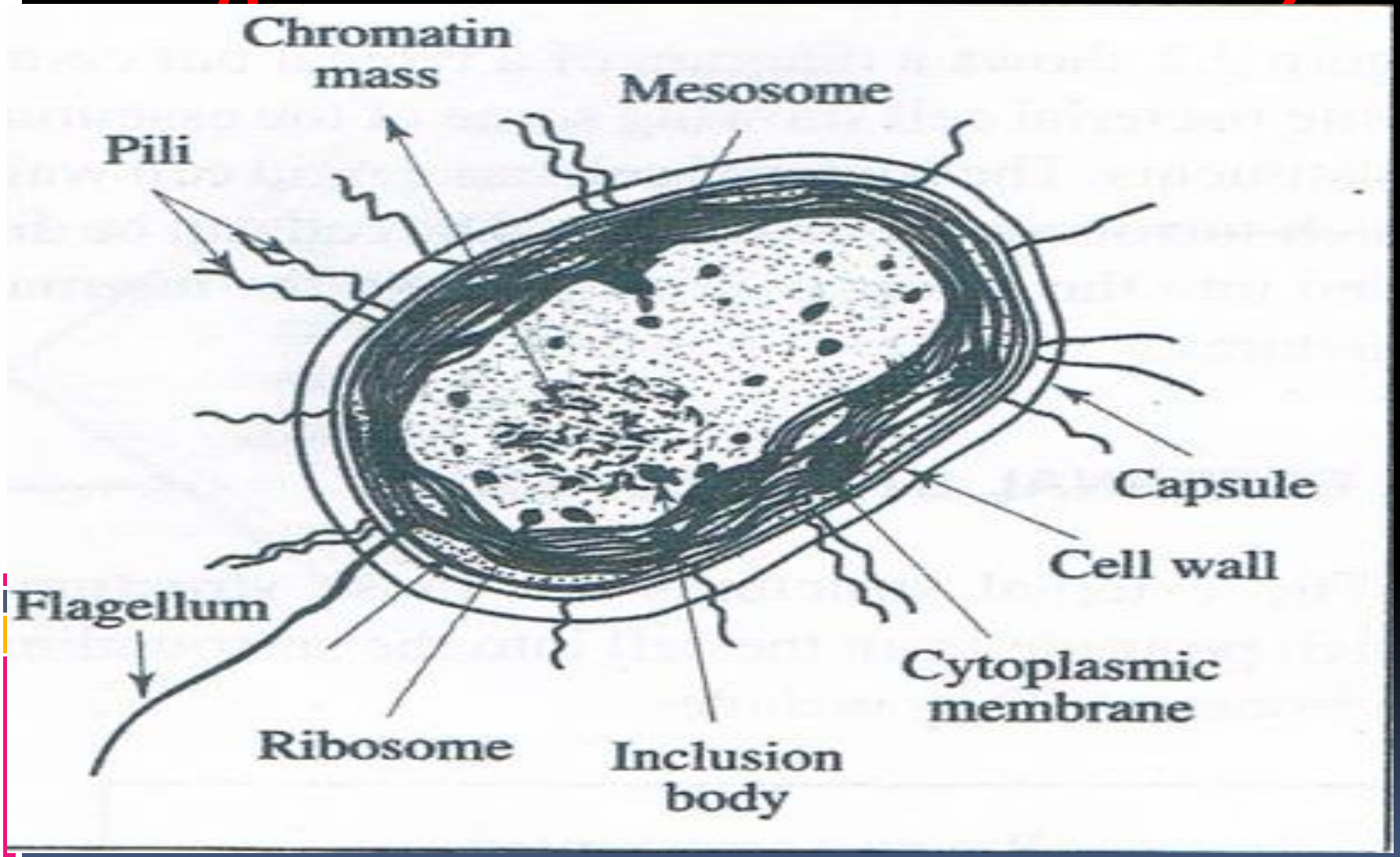
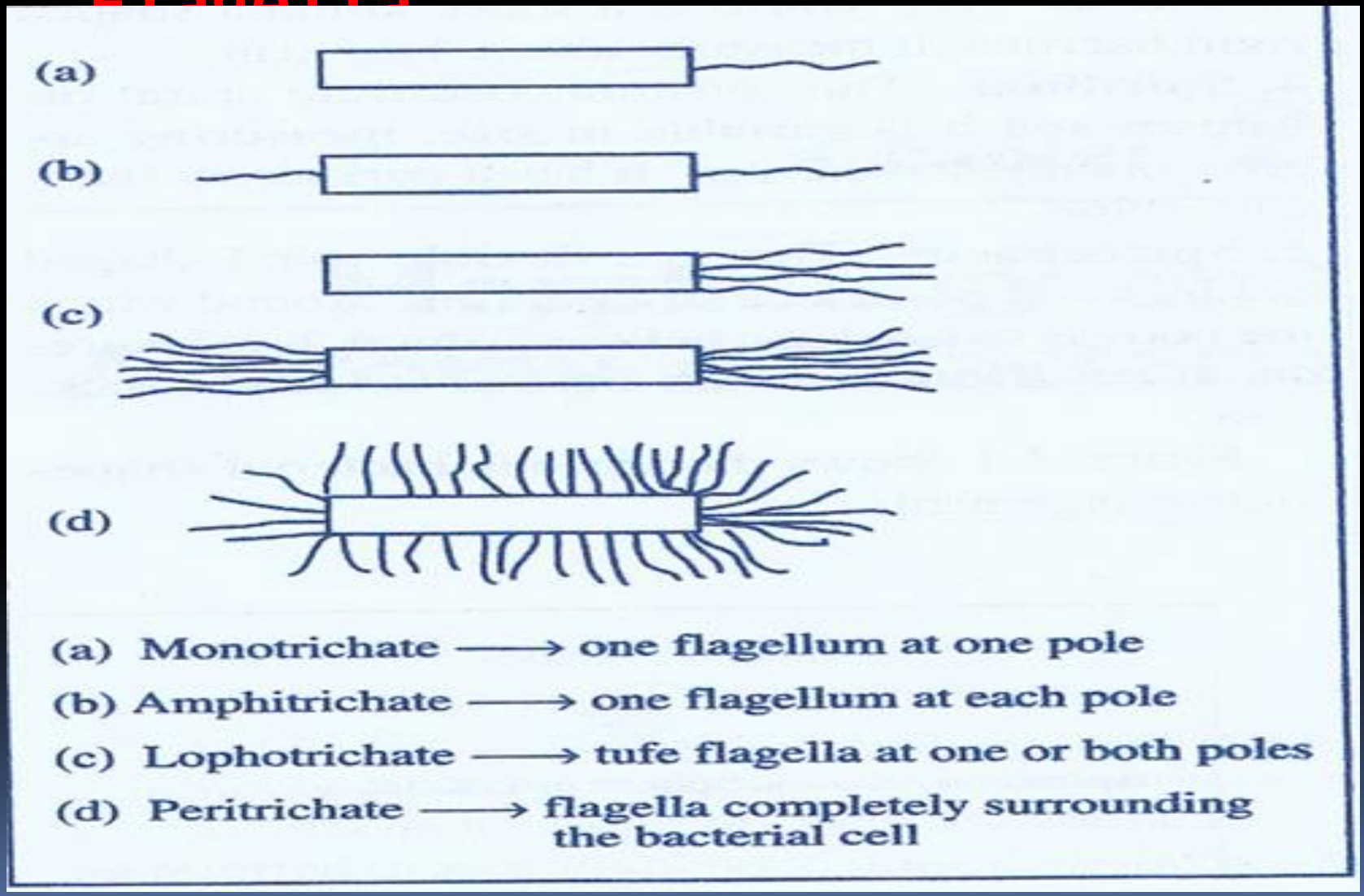


Fig. D Distribution of Flagella



1- Flagella

These are slender filaments that originate from the cytoplasm. The function as organs of motility. They are therefore seen only in organisms that are motile. They are proteins; and have characteristic patterns of arrangement on the bacteria cell (Fig. D) .
They are too thin to be seen in ordinary stained preparation s, but become visible only in sliver impregnation preparation or in electron microscopy.

2- Pili

Extruding from the cytoplasmic membrane are shorter and finer filaments than the flagella. These are the pili. Some of them are referred to as sex pili because of their role during conjugation when genes are transferred from one cell to another. Majority of them are referred to as common pili or fimbriae. These are thought to be the organs of adhesion that help bacteria to attach to the host cells.

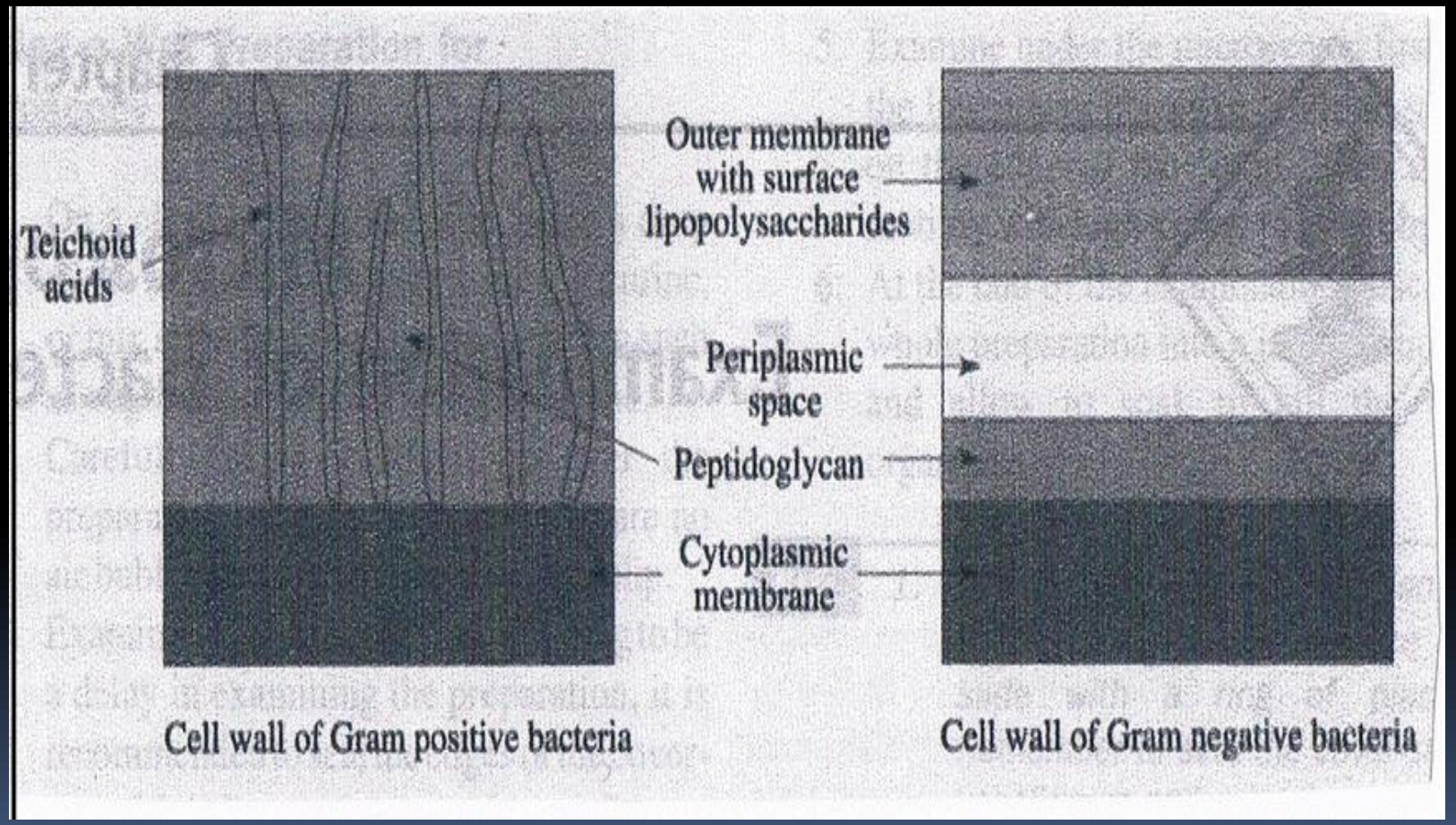
3- Capsule

This is a layer of loose slimy material which surrounds some bacterial cells. The capsule are composed mainly of polymers of polysaccharides or peptides. They resist phygocytosis and so their presence on a bacterium is associated with virulence. They are identified by negative staining due to their low affinity for simple stains.

4- Cell wall

The cell wall is a prominent distinguishing feature of the bacteria cell with a rigidity and strength that protects the cellular contents. Chemically, the cell wall consists of peptidoglycan, a mucopeptide that is composed of alternating strands of N –acetyl –glucosamine and N –acetyl –muramic acid residues, , cross –linked with peptide subunits. The peptide –glycan gives rigidity and shape to the organisms. Structurally ,the cell wall of a Gram positive bacterium differs from that of a Gram negative bacterium (Fig. E). The Gram positive cell wall is much more study than the Gram negative one due to its high content of peptidoglycan. It has neither an outer membrane that contains specific proteibs nor a preiplasmic space, both of which are present in the Gram negative cell. Teichoice acids are also part of the cell wall of Gram positive bacteria. The Gram negative cell wall has a thin
peptidoglycan layer with no teichoic acid. It has an outer membrane which consists lipopolysaccharides. The lipopolysaccharides is toxic to humans. It is called endotoxin .

Fig. E Bacterial Cell Wall



5- Cytoplasmic membrane

This is a double layer structure within the cell wall. It is composed of lipid and protein and acts as a semipermeable membrane through which nutrients pass into and out of cell.

Internal Structures

1- Mesosomes

These are convoluted imaginations within the cytoplasmic membrane. The play an important role during cell division and in the secretion of certain enzymes.

2- Nuclear Material

Within the cytoplasmic membrane, the cell itself has the " nucleus" which has no nuclear membrane and lacks definite shape. It is a single circular stand of deoxyribonucleic acid (DNA) which acts as a "nucleus" (chromosome).

3-Ribosome's

Ribosomes are located throughout the cytoplasm and are the sites of protein synthesis. They are important for conveying the genetic code of the nucleus into instructions in the manufacture of cellular components. They are composed of ribonucleic acid (RNA) and proteins

4- Cytoplasmic inclusions

There are seen in some bacteria and appear to be sources of reserved food for energy. For example, volutin (polymetaphosphate) granules associated with Corynebacteria.

5- Spores

These are dense structures produced by the bacteria, e.g., the Bacillus and Clostridium groups, that enable them to survive adverse environmental conditions. They develop within and at prises the chromosomal material surrounded by several layer of walls. The location and shape of the spore in the cell may be of diagnostic assistance e.g., the spores of Clostridium tetani and terminal, and the diameter is greater than that of the parent cell, so that they are characteristically of drum stick appearance. The positions of spores are described as terminal, sub terminal or central. Spores are resistant to heat, stains, desiccation and disinfectants. Each spore germinates to produce a vegetative cell during favorable growth conditions.

Bacteriology

Host-Parasite Relationship

3rd YEAR

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Growth, Survival, and Death of Microorganism

The population of microorganisms in the biosphere remains roughly constant because the growth of microorganisms is in turn balanced by the death of these organisms. The survival of any microbial group, within a specific niche, is ultimately influenced by successful competition for nutrients and by maintenance of a pool of living cells, often composed of host cells and a consortia of different microorganisms. Consequently, understanding competition for nutritional resources within a given micro environment is essential to understanding the growth, survival, and evolution of bacterial species (also known as physiology) Much of our understanding of microbial physiology has come from the study of isolated cultures grown under optimal conditions in laboratories (nutrient excess In the end understanding the complex interactions that ensure the survival of a specific bacterium in a microbially diverse biosphere is a matter of physiologic efficiency.

THE MEANING OF GROWTH

Growth is the orderly increase in the sum of all the components of an organism. The increase in size that results when a cell takes up water or deposits lipid or polysaccharide is not true growth. Cell multiplication is a consequence of cell division of unicellular organisms, growth leads to an increase in the number of single bacteria making up a population, referred to as a culture.

The Measurement of Microbial Concentrations

Microbial concentrations can be measured in terms of cell concentration (the number of viable cells per unit volume of culture). or of biomass concentration (dry weight of cells per unit volume of culture). These two parameters are not always equivalent because the average dry weight of the cell varies at different stages in the history of a culture. Nor are they of equal significance: In studies of microbial genetics and the inactivation of microbes, cell concentration is the significant quantity; in studies on microbial biochemistry or nutrition, biomass concentration is the significant quantity

A. Cell Concentration

The viable cell count (Table 1) is typically considered the measure of cell concentration. For most purposes, the turbidity of a culture, measured by photoelectric means, is related to the viable count in the form of a standard curve . As an alternative a rough visual estimate is sometimes possible: For example, a barely turbid suspension of Escherichia coli contains about 10^7 cells per milliliter, and a fairly turbid suspension contains about 10^8 cells per milliliter. In using turbid metric measurements, the correlation between turbidity and viable count can vary during the growth and death of a culture; cells may lose viability without producing a loss in turbidity of the culture.

TABLE 1 Example of a Viable Count

Dilution	Plate Count[a]
Undiluted	Too many to count
10^{-1}	Too many to count
10^{-2}	510
10^{-3}	72
10^{-4}	6
10^{-5}	1

[a]Each count is the average of three replicate plates.

Biomass Density

In principle, biomass can be measured directly by determining the dry weight of a microbial culture after it has been washed with distilled water. In practice, this procedure is cumbersome, and the investigator customarily prepares a standard curve that correlates dry weight with turbidity. Alternatively, the concentration of biomass can be estimated indirectly by measuring an important cellular component such as protein or by determining the volume occupied by cells that have settled out of suspension.

THE GROWTH CURVE

If a fixed volume of liquid medium is inoculated with microbial cells taken from a culture that has previously been grown to saturation and the number of viable cells per milliliter is determined periodically and plotted, a curve of the type shown in Figure A is usually obtained. The phases

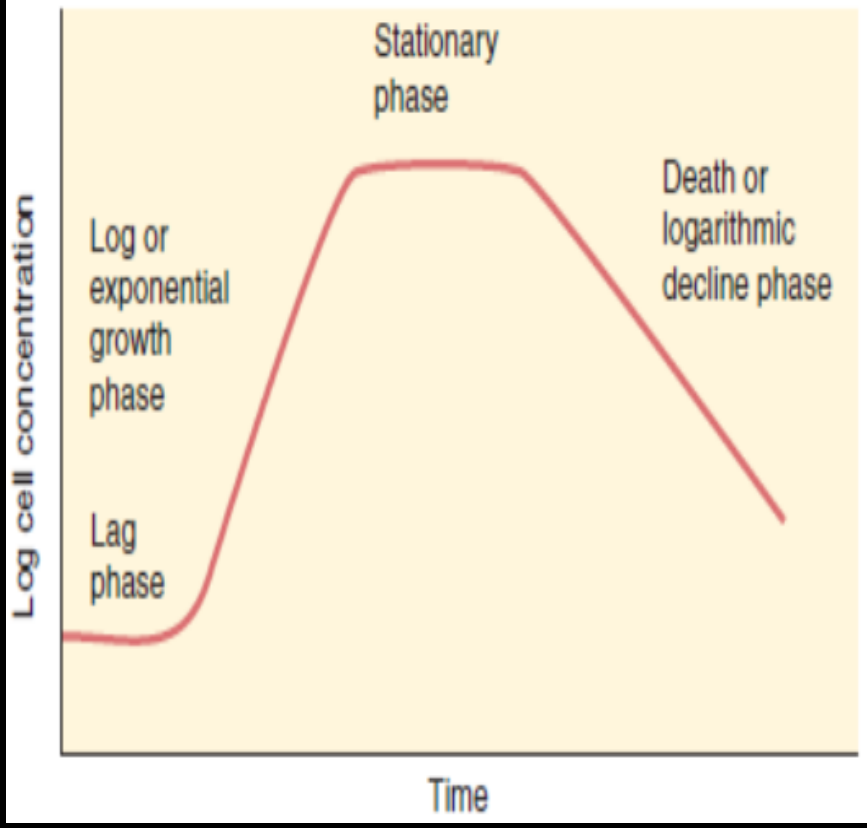

TABLE 2 Phases of the Microbial Growth Curve

Phase	Growth Rate
Lag	Zero
Exponential	Constant
Maximum stationary	Zero
Decline	Negative (death)

Figure A : a Bacterial growth curve

of the bacterial growth curve shown in Figure -A are reflections of the events in a population of cells, not in individual cells. This type of culture is referred to as a batch culture The typical growth curve may be discussed in terms of four phases (Table -2). Batch culture is a closed system with finite resources; this is very different from the environment of the human host

The Lag Phase

The lag phase represents a period during which cells, depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume. If the cells are taken from an entirely different medium , it often happens that they are genetically incapable of growth in the new medium.

The Exponential Phase

During the exponential phase, the cells are in a steady state. New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in

the medium become exhausted or toxic metabolic products accumulate and inhibit growth. For aerobic organisms, the nutrient that becomes limiting is usually oxygen. When the cell concentration exceeds about 1×10^7/mL (in the case of bacteria), the growth rate decreases unless oxygen is forced into the medium by agitation or by bubbling in air. When the bacterial concentration reaches $4\text{-}5 \times 10^9$ /mL, the rate of oxygen diffusion cannot meet the demand even in an aerated medium and growth is progressively slowed

The Maximum Stationary Phase

Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely. In most cases however, cell turnover takes place in the stationary phase: There is a slow loss of cells through death, which is balanced by the formation of new cells through growth and division. When this occurs, the total cell count slowly increases, although the viable count stays constant.

The Phase of Decline.
The Death Phase.

After a period of time in the stationary phase, which varies with the organism and with the culture conditions, the death rate increases until it reaches a steady level. In most cases, the rate of cell death is much slower than that of exponential growth. Frequently, after the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die and lyse.

A phenomenon, in which cells are called viable but not culturable (VBNC), is thought to be the result of a genetic response triggered in starving, stationary phase cells. Just as some bacteria form spores as a survival mechanism, others are able to become dormant without changes in morphology. When the appropriate conditions are available (eg, passage through an animal), VNBC microbes resume growth.

DEFINITION OF DEATH

For a microbial cell, death means the irreversible loss of the ability to reproduce (grow and divide).

Sterilization

"sterilization" as the process of killing all of the organisms in a preparation.

The Effect of Drug Concentration

When antimicrobial substances (drugs) are used to inactivate microbial cells, it is commonly observed that the concentration of drug used is related to the time required to kill a given fraction of the population by the following expression:

$$C^n t = K$$

In this equation, C is the drug concentration, t is the time required to kill a given fraction of the cells, and n and K are constants.

A. Biocide: A chemical or physical agent, usually broad spectrum, that inactivates microorganisms . Chemical biocides include hydrogen peroxide, alcohols, bleach, cycloheximide, and phenols, and physical biocides include heat and radiation. Biocides are generally broad spectrum, in contrast to anti-invectives, which have a narrower range of antimicrobial activity.

B. Bacteriostatic: A specific term referring to the property by which a biocide is able to inhibit bacterial multiplication; upon removal of the agent, multiplication resumes. (The terms "fungistatic" and "sporostatic" refer to biocides that inhibit the growth of fungi and spores, respectively.)

C. Bactericidal: A specific term referring to the property by which a biocide is able to kill bacteria. Bactericidal action differs from bacteriostasis only in being irreversible (ie, the "killed" organism can no longer reproduce even after being removed from contact with the agent). In some cases, the agent causes lysis (dissolution) of the cells; in other cases, the cells remain intact and may even continue to be metabolically active. (The terms "fungicidal," "sporicidal," and "virucidal" refer to the property whereby biocides are able to kill fungi, spores, and viruses, respectively.)

D. Sterilization: A defined process used to render a surface or product free from viable organisms, including bacterial spores.

E. Disinfectants: Products or biocides used to reduce the number of viable microorganisms, or bioburden, on or in a product or surface to a level previously specified as appropriate for its intended further handling or use. Disinfectants are not necessarily sporicidal but are sporostatic, inhibiting germination or outgrowth.

F. Septic:

Characterized by the presence of pathogenic microbes in living tissues or associated fluids.

G. Antiseptic: A biocide or product that destroys or inhibits the growth of microorganisms in or on living tissue (eg, skin) or biologic fluids (eg, mucosal secretions).

H. Aseptic: Free of, or using methods to keep free of, microorganisms.

I. Preservation: The prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods.

J. Antibiotics:

Naturally occurring and synthetically derived organic compounds that inhibit or destroy selective bacteria, generally at low concentrations.

Modes of Action

A. Damage to DNA: A number of physical and chemical agents act by damaging DNA; these include ionizing radiations, ultraviolet light, and DNA-reactive chemicals. Among the last category are alkylating agents and other compounds that react covalently with purine and pyrimidine bases to form DNA adducts or interstrand cross-links. Radiation damage DNA in several ways: Ultraviolet light, for example, induces cross-linking between adjacent pyrimidines on one or the other of the two polynucleotide strands, forming pyrimidine dimers; ionizing radiations produce breaks in single and double strands. Radiation-induced and chemically-induced DNA lesions\kill the cell mainly by interfering with DNA replication.

B. Protein Denaturation: Proteins exist in a folded, three-dimensional state determined primarily by intramolecular noncovalent interactions such as ionic, hydrophobic, and hydrogen bonds or covalent disulfide linkages. This state is called the tertiary structure of the protein; it is readily disrupted by a number of physical (eg,heat) or chemical (eg, alcohol) agents, causing the protein to become nonfunctional. The disruption of the tertiary structure of a protein is called protein denaturation.

C. Disruption of the Cell Membrane or Wall:

The cell membrane acts as a selective barrier, allowing some solutes to pass through and excluding others. Many compounds are actively transported through the membrane, becoming concentrated within the cell. The membrane is also the site of enzymes involved in the biosynthesis of components of the cell envelope. Substances that concentrate at the cell surface may alter the physical and chemical properties of the membrane, preventing its normal functions and therefore killing or inhibiting the cell The cell wall acts as a corseting structure (best characterized as a fishing net), protecting the cell against osmotic lysis. Thus, agents that destroy the wall (eg, lysozyme, which cleaves the sugar linkages) or prevent its normal synthesis (eg, penicillin, which interrupts peptidyl crosslinkages) may bring about lysis of the cell.

D. Disruption of Free Sulfhydryl Groups:

Enzymes containing cysteine have side chains terminating in sulfhydryl groups. In addition to these, coenzymes such as coenzyme A and dihydrolipoate contain free sulfhydryl groups. .Many metals such as mercuric ion likewise interfere by combining with sulfhydryls. There are sulfhydryl-containing enzymes in the cell, so oxidizing agents and heavy metals do widespread damage.

E. Chemical Antagonism:

The interference by a chemical agent with the normal reaction between a specific enzyme and its substrate is known as chemical antagonism.

Resistance to Antibacterial Agents:

The ability of bacteria to become resistant to antibacterial agents is an important factor in their control.

Physical Agents

A. Heat: Application of heat is the simplest means of sterilizing materials, provided the material is itself resistant to heat damage. A temperature of 100°C will kill all but spore forms of eubacteria within 2–3 minutes in laboratory-scale cultures; a temperature of 121°C for 15 minutes is used to kill spores. Steam is generally used, both because bacteria are more quickly killed when moist and because steam provides a means for distributing heat to all parts of the sterilizing vessel. At sea level, steam must be kept at a pressure of 15 lb/sq inches (psi) in excess of atmospheric pressure to obtain a temperature of 121°C; autoclaves or pressure cookers are used for this purpose. At higher altitudes, the pressure would need to be higher than 15 psi to reach 121°C. For sterilizing materials that must remain dry, circulating hot air electric ovens are available; because heat is less effective on dry material, it is customary to apply a temperature of 160–170°C for 1 hour or more. Under these conditions (ie, excessive temperatures applied for long periods of time), heat acts by denaturing cell proteins and nucleic acids and by disrupting cell membranes.

B. Radiation:

Ultraviolet light and ionizing radiations have various applications as sterilizing agents.

A. Alcohols

Ethyl alcohol, isopropyl alcohol, and n-propanol exhibit rapid, broad-spectrum antimicrobial activity against vegetative bacteria, viruses, and fungi but are not sporicidal. Activity is optimal when they are diluted to a concentration of 60% to 90% with water.

B. Aldehydes

Glutaraldehyde is used for low-temperature disinfection and sterilization of endoscopes and surgical equipment. It is normally used as a 2% solution to achieve sporicidal activity. Formaldehyde is bactericidal, sporicidal, and virucidal.

C. Biguanides

Chlorhexidine is widely used in handwashing and oral products and as a disinfectant and preservative. The Mycobacteria, of their unique waxy cell envelope, are generally highly resistant to these compounds.

D. Bisphenols

The bisphenols are widely used in antiseptic soaps and hand rinses. In general, they are broad spectrum but have little activity against Pseudomonas aeruginosa and molds. Triclosan and hexachlorophene are bactericidal and sporostatic.

E. Halogen-Releasing Agents

The most important types of chlorine-releasing agents are sodium hypochlorite, chlorine dioxide, and sodium dichloroisocyanurate, which are oxidizing agents that destroy the cellular activity of proteins. Hypochlorous acid is the active compound responsible for the actericidal and virucidal effect of these compounds. At higher concentrations, this group is sporicidal. Iodine is rapidly bactericidal, fungicidal,

F. Heavy Metal Derivatives

Silver (Ag+) sulfadiazine, a combination of two antibacterial agents, Ag+ and sulfadiazine, has a broad spectrum of activity. Binding to cell components such as DNA may be responsible\ for its inhibitory properties.

G. Organic Acids

Organic acids are used as preservatives in the pharmaceutical and food industries. Benzoic acid is fungistatic; propionic acid is both bacteriostatic and fungistatic.

H. Peroxygens

Hydrogen peroxide has broad-spectrum activity against viruses, bacteria, yeasts, and bacterial spores. Sporicidal activity requires higher concentrations (10–30%) of H_2O_2 and longer contact times.

I. Phenols

Phenol and many phenolic compounds have antiseptic, disinfectant, or preservative properties.

J. Quaternary Ammonium Compounds(QACs),

These compounds have two regions in their molecular structures, one a water-repelling (hydrophobic) group and the other a water-attracting (hydrophilic) group. Cationic detergents.

K. Vapor-Phase Sterilants

Heat-sensitive medical devices and surgical supplies can be effectively sterilized by vapor-phase systems using ethylene oxide, formaldehyde, hydrogen peroxide, or peracetic acid.

Cultivation of Microorganisms

Cultivation is the process of propagating organisms by providing the proper environmental conditions. Growing microorganisms are making replicas of themselves, and they require the elements present in their chemical composition. Nutrients must provide these elements in metabolically accessible form.

REQUIREMENTS FOR GROWTH

Most of the dry weight of microorganisms is organic matter containing the elements carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. In addition, inorganic ions such as potassium, sodium, iron, magnesium, calcium, and chloride are required to facilitate enzymatic catalysis and to maintain chemical gradients across the cell membrane. For the most part, the organic matter is in macromolecules formed by anhydride bonds between building blocks. Synthesis of the anhydride bonds requires chemical energy, which is provided by the two phosphodiester bonds in ATP (adenosine triphosphate).

SOURCES OF METABOLIC ENERGY

The three major mechanisms for generating metabolic energy are fermentation, respiration, and photosynthesis. At least one of these mechanisms must be used if an organism is to grow.

ENVIRONMENTAL FACTORS AFFECTING GROWTH

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as(1) pH, temperature, (2) carbon source, about 1 g/L; (3) nitrogen source, about 1 g/L; (4) minerals: sulfur and phosphorus, about 50 mg/L of each, and trace elements, 0.1–1 mg/L of each; (5) growth factors: amino acids, purines, and pyrimidines, about 50 mg/L of each, and vitamins, 0.1–1 mg/L of each

CULTIVATION METHODS

A. Growing Cells of a Given Species

Microorganisms observed microscopically to be growing in a natural environment may prove exceedingly difficult to grow in pure culture in an artificial medium.

B. Microbiologic Examination of Natural Materials

A given natural material may contain many different microenvironments, each providing a niche for a different species.

C. Isolation of a Particular Type of Microorganism

A small sample of soil, if handled properly, will yield a different type of organism for every microenvironment present. For fertile soil (moist, aerated, rich in minerals and organic matter

Isolation of Microorganisms in Pure Culture

To study the properties of a given organism, it is necessary to handle it in pure culture free of all other types of organisms.

Normal Human Microbiota

The term "normal microbial flora" denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. The microorganisms that live inside and on humans (now referred to as the normal microbiotal) are estimated to outnumber human somatic and germ cells by a factor of 10. The genomes of these microbial symbiosis are collectively defined as the microbiome . Research has shown that the " normal microbiotal " provides a first line of defense against microbial pathogens, assist in digestion, play a role in toxin degradation, and contribute to maturation of the immune system. Shift s in the normal microbiota or stimulation of inflammation by these commensals may cause diseases such as inflammatory bowel disease.

HUMAN MICROBIOME PROJECT

In a broad attempt to understand the role played by resident microbial ecosystems in human health and disease , the microbiome, and the factors that influence the distribution and evolution of the constituent microorganisms.

ROLE OF THE RESIDENT MICROBIOTA

The skin and mucous membranes always harbor a variety of microorganisms that can be arranged into two groups:
(1) the resident microbiota consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself;

2) the transient microbiota consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks. The transient microbiotal is derived from the environment, does not produce disease, and does not establish itself permanently on the surface. Members of the transient microbiotal are generally of little significance so long as the normal resident flora remains intact. However, if the resident microbiotal is disturbed, transient microorganisms may colonize, proliferate, and produce disease. other parts of the body. Such organisms behave as opportunists and may become pathogens. On the other hand, members of the normal microbiotal may themselves produce disease under certain circumstances. These organisms are adapted to a noninvasive mode of life defined by the limitations of the environment

. If forcefully removed from the restrictions of that environment and introduced into the bloodstream or tissues, these organisms may become pathogenic. For example, streptococci of the viridans group are the most common resident organisms of the upper respiratory tract. If large numbers of them are introduced into the bloodstream (eg, after tooth extraction or oral surgery), they may settle on deformed or prosthetic heart valves and produce infective endocarditis. Small numbers occur transiently in the bloodstream with minor trauma(eg, dental scaling or vigorous brushing). Bactericides species are the most common resident bacteria of the large intestine and are quite harmless in that location. However, if introduced into the peritoneal cavity or into pelvic tissues along with other bacteria as a result of trauma,

they cause suppuration and bacteremia. There are many other examples, but the important point is that the normal resident microbiotal is harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. They may produce disease if introduced into foreign locations in large numbers and if predisposing factors are present.