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Topics:

1. **Systematic Classification of Bacteria and Characteristics of Major Group – Taxonomy**
2. **Protoplasts, Spheroplasts and L-Forms**

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SYSTEMATIC CLASSIFICATION OF BACTERIA AND CHARACTERISTICS OF MAJOR GROUP – TAXONOMY

What is Taxonomy and Bacterial systematics?

Taxonomy (from the Greek words *taxis* = arrangement or order, and *nemein* = to distribute or govern) is the science of the classification of various living organisms, which in the case of bacteria, consist of three independent, but interrelated disciplines, namely **classification**, **nomenclature** and **identification**. It is a means by which bacteria can be grouped together. Bacteria having similarities with respect to the criteria used are in the same group, and are separated from the other groups of microorganisms that have different characteristics. It is the science of biological classification.

The classification is the arrangement of organisms into groups (taxa), while nomenclature refers to the assignment of names to taxonomic groups, and identification refers to the determination of the particular taxon to which a particular isolate belongs.

Bacterial systematics is a field, which is frequently used synonymously with taxonomy, however, the scope of systematics is much broader, including data on bacterial morphology, physiology, molecular biology and biochemistry, metabolic products, pathogenic potential, ecological niches and epidemiology to characterize, arrange and classify bacteria. It is the study of organisms (bacteria) with the ultimate objective of characterizing and arranging them in an orderly manner.

It is important to note that the advent of molecular biological methods and sequencing is leading a revolution with regards to the reporting of novel taxa and changes in the taxonomy of already described bacterial species.

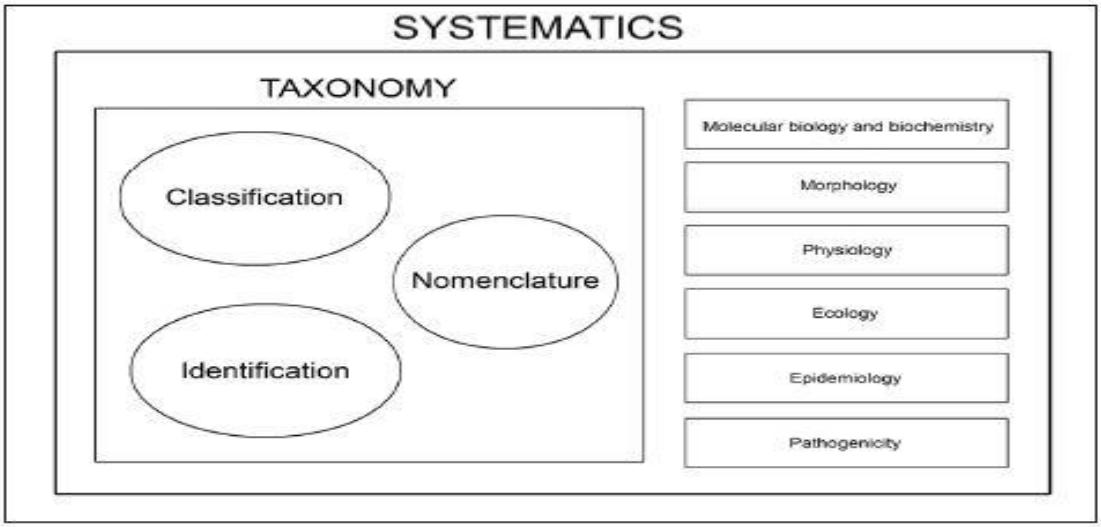

Figure 1: Relationship of the field of taxonomy and bacterial systematics.

Nomenclature

The discipline of *nomenclature* is mainly concerned with the assignment of names to taxonomic units or groups, on the basis of specific rules. Before a name could be designated for any microorganism, one has to describe its biological characteristics (for its future identification), allowing for its classification in the subordinate system. Nomenclature is the set of rules and conventions which govern the names of taxa. Each name has two components – the Generic name and the specific epithet. This system of providing a name with two components is called Binomial nomenclature. This naming system was given by Carolus Linnaeus and is being practised by biologists all over the world. Example is *Escherichia coli*,

in this name, '*Escherichia*' represents the genus while '*coli*', is a particular species, or a specific epithet. Other universal rules of nomenclature are as follows:

- Biological names are generally in Latin and written in italics. They are Latinised or derived from Latin irrespective of their origin.
- The first word in a biological name represents the genus while the second component denotes the specific epithet.
- Both the words in a biological name, when handwritten, are separately underlined, or printed in italics to indicate their Latin origin.
- Names of categories at or above the genus level may be used alone, but species and subspecies names (species names) may not. In other words…never use a species name alone.
- The first word denoting the genus starts with a capital letter while the specific epithet starts with a small letter.
- When to use Initials - A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Be careful with the "S" words; Salmonella, Shigella, Serratia, Staphylococcus, Streptococcus, etc.
- Common Names - Vernacular (common) names should be in lowercase roman type, nonitalic (e.g., streptococcus, brucella). However when referring to the actual genus name (or above) always capitalize and italicize.
- Subspecies and Serovars - For Salmonella, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. arizonae at first use, *S. enterica* subsp. arizonae thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: Salmonella serovar Typhimurium.
- Abbreviations for Species – use "sp." for a particular species, "spp." for several species ("spp" stands for "species plural"). These abbreviations are not italicized; e.g. *Clostridium* sp. or *Clostridium* spp.
- Names of all taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be underlined if handwritten; strain designations and numbers are not. If all the surrounding text is italic, then the binary name would be non-italic (Roman typeface) or underlined (e.g. A common cause of diarrhea is *E. coli* 0157, a gram negative bacillus).
- If need be, the name of the author appears after the specific epithet, i.e., at the end of the biological name and is written in an abbreviated form, e.g., *Mangifera indica* Linn. It indicates that this species was first described by Linnaeus.

Today, microorganism names originate from four different sources:

1. **Descriptive** – For example *Staphylococcus aureus* (grape-like cluster of spheres, golden in color), *Streptococcus viridans* (chains of spheres, green in colony color), *Proteus vulgaris* (first and common), *Helicobacter pylori* (spiral shaped rod at the entrance to the duodenum).
2. **Scientist's names** – e.g., *Escherichia coli* (Theodor Esherich), *Erlichia* (Paul Erlich), *Nessieria* (Albert Neisser), *Listeria* (Joseph Lister), *Pasturella* (Louis Pasteur), *Yersinia* (AlexandreYersin), *Bartonella* (Alberto Barton), *Morganella* (H. de R. Morgan), *Edwardsiella* (P. R. Edwards).

3. **Geographic places** – e.g., *Legionella longbeachiae* (Long Beach, California), *Pasturella tularensis* (Tulare County, California), *Pseudomonas fairmontensis* (Fairmount Park, Pennsylvania), *Mycobacterium genavense* (Geneva, Switzerland), *Blastomyces brasiliensis* (Brazil), *Providencia spp*. (Brown University, Providence, RI).
4. **Organizations** – e.g., *Legionella* (American Legion), *Afipia felis* (Air Force Institute of Pathology), *Cedecea spp.* (Centers for Disease Control), *Bilophila wadsworthia* (VA Wadsworth Medical Center in Los Angeles).

Classification

Classification refers to the arrangements of bacteria into groups or taxa (sing, taxon) on the basis of their mutual similarity or evolutionary relatedness. There different criteria for the classification of bacteria. These include;

- **Morphological characteristics** - Bacteria are grouped according to their phenotypic characteristics ranging from cell shape, Cell size, Cilia and flagella, Cellular inclusions, Colour, Endospore shape and location, Spore morphology and location, Colonial morphology, to Staining behaviour. Morphological characteristics play important role in microbial classification and identification due to following reasons:
    - They are easily studied and analysed especially in eukaryotic microorganisms and comparatively complex prokaryotes.
    - They normally do not vary greatly with environmental changes as they are resulted in by the expression of many genes and, therefore, are usually genetically stable.
    - Morphological similarity amongst microorganisms often is a good indication of phylogenetic relatedness.
- **Physiological, metabolic characteristics and ecological characteristics –** Some physiological and metabolic characteristics are very useful in classifying and identifying microorganisms because they are directly related to the nature and activity of microbial enzymes and transport proteins. Ecological characteristics, i.e., the characteristics of relationship of microorganisms to their environment significantly contribute in microbial taxonomy. It is because even very closely related microorganisms may vary considerably with respect to their ecological characteristics. They involve the Carbon and nitrogen sources, Cell wall constituents, Energy sources, Fermentation products, Motility, Osmotic tolerance, Storage inclusions, General nutritional type, Growth temperature optimum and range, pH optimum and growth range, Photosynthetic pigments, Salt requirements and tolerance, Secondary metabolites formed, Sensitivity to antibiotics.
- **Biochemical characteristics -** Fermentation of carbohydrates, Hydrolysis of starch and cellulose, Production of indole, hydrogen sulphide, acetyl methyl carbonol, etc. in media, Reduction of Nitrate, Sulphate, Methylene blue or Litmus in media, Production of specific enzymes (Phosphatse, Hyaluronidase, Cellulase etc.).
- **Serological method -** Use group specific antiserum isolated from the plasma of organism that have been sensitized to the organism.
    - The antiserum contains antibody proteins that react with antigens on the unknown organism.
    - The reaction can be detected by examining agglutination or by using sera labelled with fluorescent labels.
- **Genetic analysis -** Genetic analysis is mostly used in the classification of eukaryotic microorganisms because the species is defined in these organisms in terms of sexual reproduction which occurs in them. This analysis is sometimes employed in the

classification of prokaryotic microorganisms particularly those that use the processes of conjugation and transformation for gene exchange.
    - The study of transformation and conjugation and Transduction in bacteria.
    - Extrachromosomal elements such as Plasmid, Transposon etc.
    - Life cycle i.e. lytic cycle and Lysogenic cycle.
- **Molecular characteristics -** Some recent molecular approaches such as genomic DNA GC ratios, nucleic acid hybridization, nucleic acid sequencing, ribotyping, DNA fingerprinting, 16 S rDNA sequence analysis, and comparison of proteins have become increasingly important and are used routinely for determining the characteristics of microorganisms to be used in microbial taxonomy.

Identification

Identification represents the practical side of taxonomy, which is the process of determining that a particular isolate belongs to a recognized taxon. It is the practical use of classification criteria to distinguish certain organisms from others, in order to verify the authenticity or utility of a strain or a particular reaction, or to isolate and identify the organism that causes a disease.

Importance of taxonomy

- Permits the organization to organize huge amounts of information about organism.
- Allows predictions and hypotheses for further research based on knowledge of identical bacteria.
- Places organisms in useful groups with precise names that permit effective communication between investigators.
- Essential for the identification of organisms. In the area of clinical microbiology. Treatment of bacterial disease often become exceptionally difficult if the pathogen is not properly identified.

The Major Taxonomic Groups and Hierarchy

Generally and ancestrally, there are five (5) kingdom system including Monera, Protista, Plantae, Fungi, and Animalia and three (3) domain system including Bacteria, Archaea, and Eukaryotes, as shown in the figure below.

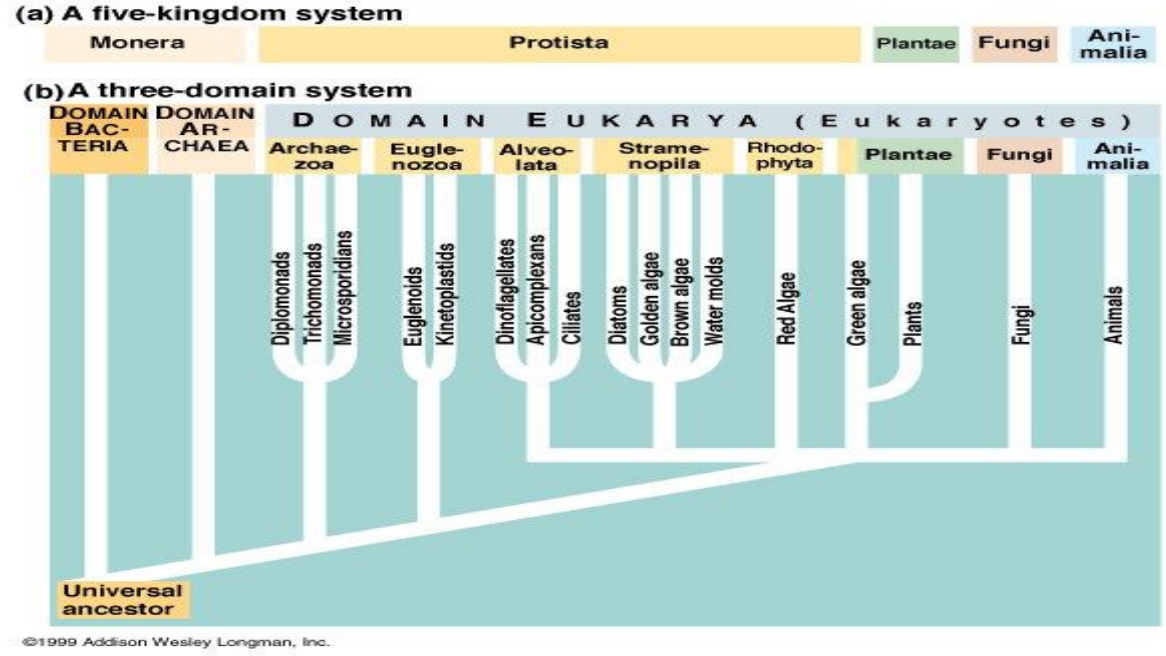

Figure 2: Kingdom and Domain classification.

For the Kingdoms, Kingdom Monera includes microscopic, single celled organisms with cell wall, no proper nucleus e.g. all bacteria. Kingdom Protoctista (Protista) includes single celled organisms with well-formed nucleus e.g. Amoeba, malarial parasite, Chlamydomonas. Kingdom FUNGI includes multicellular or many celled organisms. The body is made of network (mycelium) of fine threads called hyphae. Fungi feed on dead decaying matter (saprotrophs) e.g. Mushroom, yeast, bread mould. Kingdom Plantae includes: Multicellular eukaryotes with cellulose cell wall and chlorophyll present in their cells and Autotrophic and thus carry out photosynthesis. Kingdom Animalia includes organisms with the following characteristics; Multicellular, eukaryotes. Heterotrophic, so feed on plants or other animals, Possess special organs for locomotion or movement from one place to another.

Table 1: Characteristic features of the five kingdoms.

Major criteria on which five-kingdom classification is based

	KINGDOM				
CRITERION	MONERA	PROTISTA	PLANTAE	FUNGI	ANIMALIA
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cellular organization	Unicellular	Unicellular	Multicellular	Multicellular	Multicellular
Nutrition mode	Variable-phototrophic/heterotrophic/ chemoautotrophic	phototrophic/heterotrophic	Autotrophic (photosynthesis)	Heterotrophic (Absorption)	Heterotrophic (ingestion)
Reproduction	Asexual	Asexual or sexual without embryo stage	Asexual or sexual with or without embryo stage	Asexual or sexual with spore	Sexual with embryo stage
Ecological role	Variable	Variable	Producer	Decomposer	Consumer

The domain Archaebacteria can live in extremely harsh environments. They do not require oxygen and can live in extremely salty environments as well as extremely hot environments. Domain Eukarya contains all of the eukaryotes (organisms with a nucleus in their cells) including Protista, Fungi, Plantae, and Animalia. Domain Eubacteria "true bacteria" are the kind of bacteria likely to make us sick, live in our gut to help us digest food, or be used in the making of cheese.

Table 2: Characteristic features of the three domains.

Characteristic features of three domains.

CHARACTER	BACTERIA	ARCHAEA	EUKARYA
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
Cell wall	Present; contain peptidoglycan	Present; peptidoglycan absent	Present/absent; peptidoglycan absent
Membrane lipids	Diacyl glycerol, diesters	isoprenoid ,glycerol, diethers or diglycerol tetraethers	Glycerol , fattyacyl diesters
Genetic material	Small circular DNA not associated with histones	Small circular DNA associated with histones like proteins	Large linear DNA associated with histones
Translation (first amino acid)	Formylmethionine	Methionine	Methionine
RNA polymerase	One; simple	One; complex	Three; complex

The most basic taxonomic group (i.e. unit) in bacterial taxonomy is the species, while groups of species are collected into genera (genus), which are then collected into families (*Familia*), families into orders (*Ordo*), orders into classes (*Classis*), classes into phyla (*Phylum*) and phyla into a domain (or *Kingdom*, the highest level), however, there are subgroups to these main classifications. Groups of bacteria at each rank or level have names with endings or suffixes characteristic to that rank or level. Nevertheless, taxonomic units under species may still be relevant (especially in the case of medically-relevant bacteria), because members among specific species can be distinguished on the basis of certain biological or genetic characteristics: these members may be classified in a sub-group of members, called *subspecies*. An example for this is the differentiation of *Camplyobacter* species*: C. fetus subsp.* veneralis is a causative agent of sexually transmitted diseases and miscarriage among cattle, while *C. fetus subsp.* fetus may cause intrauterine infection in humans. Antigenic characteristics may be another possible way to distinguish subgroups under the threshold of species, called *serogroups* or *serovariants*.

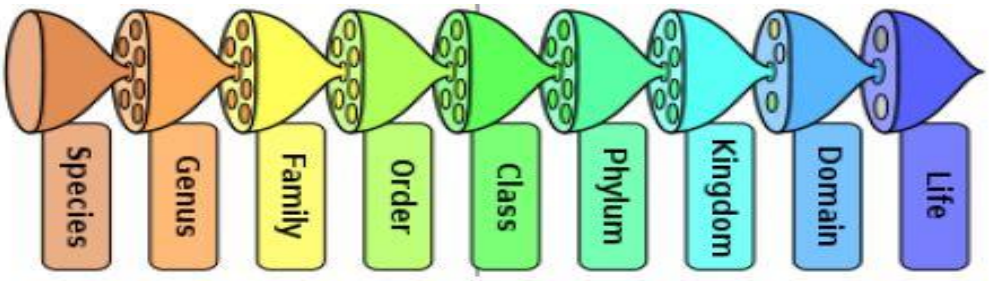

Figure 3: The hierarchy of biological classifications of eight major taxonomic.

Species
Taxonomic studies consider a group of individual organisms with fundamental similarities as a species. One should be able to distinguish one species from the other closely related species based on the distinct morphological differences.

Genus
Genus comprises a group of related species which has more characters in common in comparison to species of other genera. We can say that genera are aggregates of closely related species.

Family
The next category, Family, has a group of related genera with still less number of similarities as compared to genus and species.

Order
You have seen that categories like species, genus and families are based on a number of similar characters. Generally, order and other higher taxonomic categories are identified based on the aggregates of characters. Order being a higher category, is the assemblage of families which exhibit a few similar characters. The similar characters are less in number as compared to different genera included in a family.

Class
This category includes related orders.

Phylum
Classes comprising animals like fishes, amphibians, reptiles, birds along with mammals constitute the next higher category called Phylum. There are 23+ bacterial phyla

Domain/Kingdom
Every organism belongs to one of the five kingdoms and 3 domains of life.

Table 3: Example of taxonomic classification for a common Gram-positive, Gram-negative and an atypical pathogen

	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Mycoplasma pneumoniae***
Kingdom	Bacteria	Bacteria	Bacteria
Phylum	Firmicutes	Proteobacteria	Tenericutes
Class	Bacilli	Gammaproteobacteria	Mollicutes
Order	*Bacillales*	*Pseudomonadales*	*Mycoplasmatales*
Family	*Staphylococcaceae*	*Pseudomonadaceae*	*Mycoplasmataceae*
Genus	*Staphylococcus*	*Pseudomonas*	*Mycoplasma*
Species	*S. aureus*	*P. aeruginosa*	*M. pneumoniae*

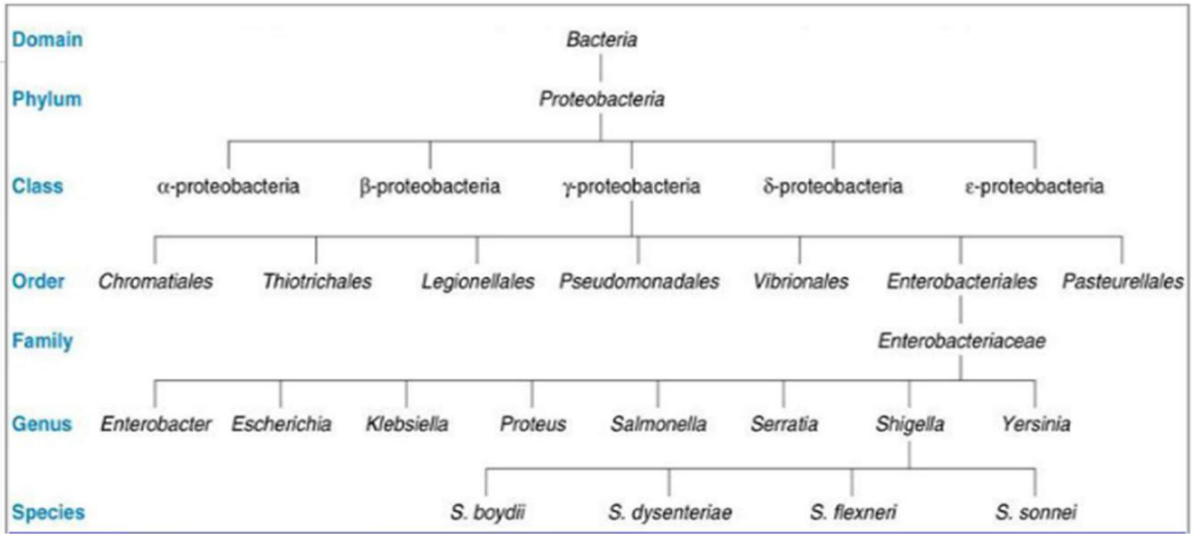

Figure 4: Example of taxonomic classification for *Shigella spp*

Characteristics of the Kingdom Classifications

Monera (Archaebacteria, Bacteria, and Cyanobacteria)
1. It is the kingdom of all the prokaryotes and includes eubacteria, cyanobacteria (blue-green algae) and archebacteria.
2. The organisms are unicellular, colonial, mycelial and filamentous in form.
3. They lack true nuclei and other membrane bound organelles such as mitochondrion, chloroplast, Golgi bodies, lysosomes etc. and DNA, which is the genetic material and is called nucleoid, is not found associated with histone proteins; cell wall is often present but chemically made up material other than cellulose.
4. Mode of nutrition varies from autotrophy to heterotrophy.
5. Sexual reproduction is absent and asexual reproduction may take place through fission, fragmentation, budding and sporulation.

Protista (Phytoplanktonic Algae, Protozoa, and Slime Moulds)

1. It is a group of organisms differing widely with one another except that they all are simple and minute eukaryotes. It includes microalgae, protozoa and slime moulds.
2. Majority of them are unicellular but some may be colonial in form.
3. They contain true nuclei and membrane bound organelles; cell wall may or may not be present.
4. Nutrition is much diversified and may be autotrophic (via photosynthesis) or heterotrophic (ingestion/absorption).
5. Asexual means of reproduction is common but when organisms reproduce sexually, embryo is not formed.

Fungi (The Fungi)

1. It is the group of mostly multicellular or multinucleate achlorophyllous and spore-producing eukaryotic organisms and includes mildews, moulds, yeasts, morals, truffles, mushrooms, rusts etc.
2. The body of organisms is mycelial in form; cell wall is present and made up of chitin or cellulose.
3. Nutrition is absorptive heterotrophy where organism secretes digestive enzymes into the substrate and then absorbs the digested food.
4. Asexual reproduction is primary mode of reduction and sexual reproduction causes formation of specialized spores.
5. They play the ecological role of decomposer.

Plantae (Macroalgae and Plants)

1. It includes all coloured multicellular photosynthetic eukaryotic organisms commonly called as plants. The important constituents are macroalgae, bryophytes, pteridophytes, gymnosperms and angiosperms.
2. Plant body is either thalloid (algae and some of bryophytes) or differentiated into root, stem and leaves; non-motile; Cell wall is present and it is chemically made up of cellulose.
3. Nutrition by: autotrophy (photosynthetic)
4. Both asexual and sexual reproductions occur. An embryo stage is present except in algal group.
5. They play the ecological role of producers.

Animalia (Invertebrate and Vertebrate Animals)

1. It is a group of all macroscopic animals derived from zygote and includes sponges, coelentrates, worms, annelids, arthopodes, mollusces, star fishes, fishes, amphibians, reptiles, birds and mammals.
2. Organisms are multicellular with higher degree of body organization where tissue differentiation usually leads to specialized organ formation. Eukaryotic cell is without cell wall and chlorophyll pigments.
3. They exhibit mobility, sensitivity to different stimuli and definite growth.
4. They reproduce primarily by sexual reproduction and embryo stage is usually present. 5. They play ecological role of consumer.

PROTOPLASTS, SPHEROPLASTS AND L-FORMS

L-forms

The peptidoglycan (PG) cellwall is one of the defining structures of bacterial cells. The genes encoding the enzymes for its synthesis are conserved and are present in all major bacterial lineages, suggesting that the wall emerged very early in evolution, and it may have been pivotal in enabling the early bacterial radiation. The wall is a crucial determinant of bacterial cell shape. It is an elastic structure that confines the cell membrane, counteracting the outward osmotic pressure and enabling the maintenance of turgor in the cell structure. The wall is also crucial for cell division, and many components of the FtsZ-dependent cell division machinery, which is again conserved virtually throughout the bacteria, are concerned with synthesis of cell wall material at the division site. The wall is an important target for antibiotics, such as b-lactams and glycopeptides, and fragments of the wall are recognized by innate immune receptors, helping to trigger powerful immune responses to infection.

Despite the evident importance of the wall, some groups of bacteria do not possess the genes for wall synthesis. The formation of the structures without cell wall can occur naturally. Such bacteria are referred to as L-forms. L-form bacteria, also known as L-phase bacteria, L-phase variants, and cell wall-deficient (CWD) bacteria, are strains of bacteria that lack cell walls. They are described as variants of normally walled bacteria that have adapted to grow in the complete absence of cell wall synthesis. They were first isolated in 1935 by Emmy Klieneberger-Nobel, who named them "L-forms" after the Lister Institute in London where she was working. This has important physiological and genetic consequences for the wide range of bacteria that can carry out this switch, including loss of regular shape, osmotic sensitivity, resistance to many wall-targeting antibiotics and ability to tolerate complete deletion of genes involved in PG synthesis and of the FtsZ-based cell-division apparatus. Two types of L-forms are distinguished: unstable L-forms that are capable of dividing, but can revert to the original morphology and stable L-forms that are unable to revert to the original bacteria. Examples of bacterial genera that can produce L-forms include *Bacillus*, *Clostridium*, *Haemophilus*, *Pseudomonas*, *Staphylococcus*, and *Vibrio*. The majority of L-forms require osmoprotective conditions for growth, which can be achieved by addition of osmolytes, typically, sucrose or salt, to culture media. L-form growth may also be promoted by other media components, such as magnesium or serum. For unknown reasons, L-forms tend to grow more robustly on solid or semi-solid media.

Appearance and Cell Division

Bacterial morphology is determined by the cell wall. Since the L-form has no cell wall, its morphology is different from that of the strain of bacteria from which it is derived. Typical L-form cells are spheres or spheroids. For example, L-forms of the rod-shaped bacterium *Bacillus subtilis* appear round when viewed by phase contrast microscopy or by transmission electron microscopy. Although L-forms can develop from Gram-positive as well as from Gram-negative bacteria, in a Gram stain test, the L-forms always colour Gram-negative, due to the lack of a cell wall. The cell wall is important for cell division, which, in most bacteria, occurs by binary fission. This process usually requires a cell wall and components of the bacterial cytoskeleton such as FtsZ. The ability of L-form bacteria to grow and divide in the absence of both of these structures is highly unusual, and may represent a form of cell division that was important in early forms of life. This novel mode of division seems to involve the extension of thin protrusions from the cell's surface and these protrusions then pinching off to form new cells. The lack of cell wall in L-forms means that division is disorganised, giving rise to a variety of cell sizes, from very tiny to very big.

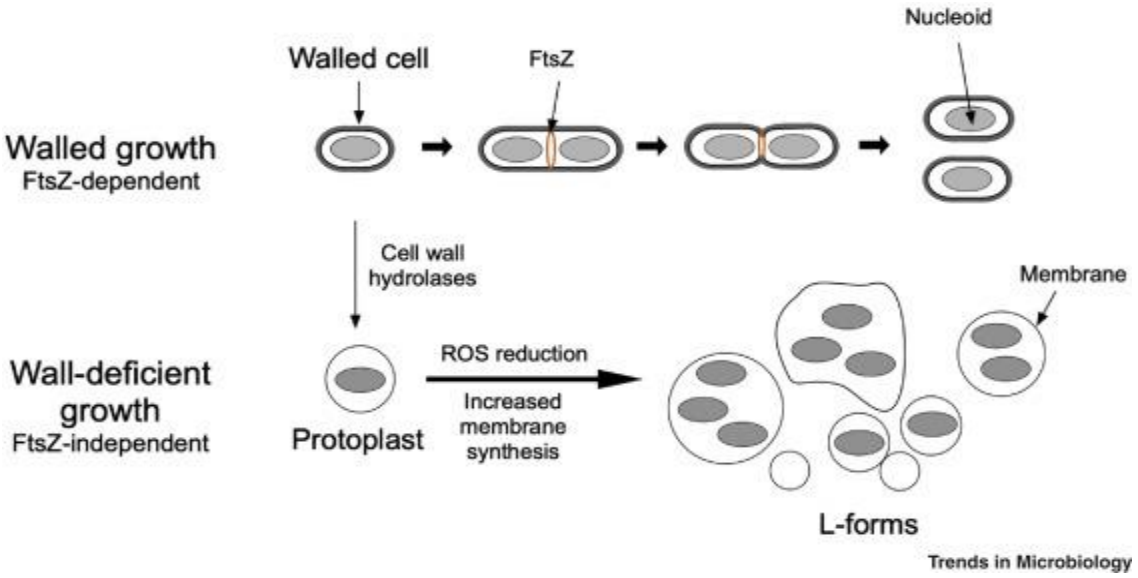

Figure 5: Walled growth vs Wall-deficient growth.

Protoplasts and Spheroplasts

Over the years, researchers realized that the L-form state can be induced experimentally in many bacterial species by treatment of cells with antibiotics, lytic enzymes and/or certain amino acids, which interfere with the bacterial cell wall or its synthesis. Cells treated in these ways to remove the cell wall are called protoplasts or spheroplasts and are operationally distinguished from L-forms by their inability (unlike L-forms) to grow and proliferate indefinitely.

Both protoplasts and spheroplasts refer to altered forms of plant, bacterial or fungal cells from which the cell wall (the principal shape-maintaining structure) has been partially or completely removed. These cells usually have all the other cellular components, except for the cell wall. When used in reference to bacterial cells, protoplasts may also refer to the spherical shape assumed by gram-positive bacteria while spheroplasts refer to the spherical shape assumed by gram-negative bacteria upon partial or complete removal of the cell wall. Cells with compromised cell walls assume a characteristic spherical shape to better withstand the rigors of its surrounding environment. They are also extremely sensitive to osmotic and mechanical shock. Another main difference between protoplasts and spheroplasts is the number of membranes present. Protoplasts are bounded by a single membrane while spheroplasts have two - an inner membrane and an outer membrane. It is also possible to generate a gram-negative protoplast by the removal of the outer membrane. Thus, in essence, protoplast refers to a bacterial sphere that is bounded by a single membrane and spheroplast refers to a sphere that is bounded by two membranes.

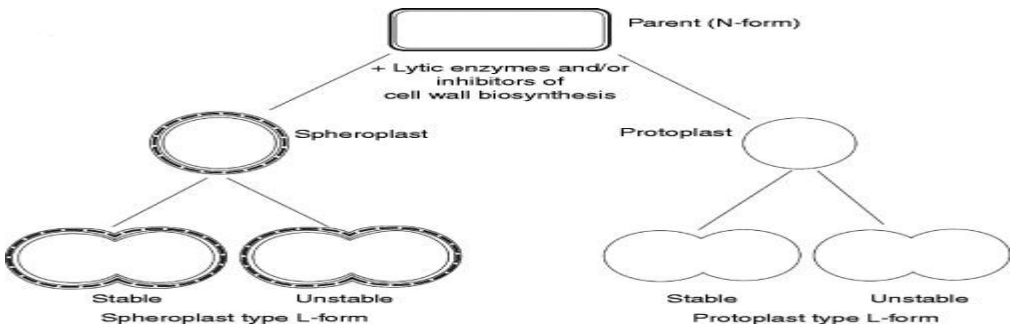

Figure 6: Protoplasts vs Spheroplasts.

Bacteria are induced to form protoplasts or spheroplasts typically by laboratory manipulation. Since the cell walls are composed of a variety of polysaccharides, viable protoplasts can be prepared by weakening the primary stress-bearing layer of the cell wall (peptidoglycan) using the appropriate enzymes. To accomplishing this purpose, researchers may either use mechanical or enzymatic methods.

Mechanical Method

In the past, protoplasts were isolated using mechanical methods which were labour-intensive, tedious and inefficient. Additionally, these methods were restricted to certain tissues with vacuolated cells and produced extremely low yields and barely viable protoplasts. For these reasons, most researchers now prefer the enzymatic method, except in cases where the enzymes can have a damaging effect on the resulting protoplasts.

Enzymatic Method

For plant cells, cellulase, pectinase, and xylanase can be used to break down the cell walls while lysosome (+EDTA) can be used to produce protoplasts from gram-positive bacteria. For fungal cells, using chitinase will do the trick. Similarly, spheroplasts can be prepared from gram-negative bacteria using procedures similar to those used in preparing protoplasts.

The process of creating protoplasts and spheroplasts must be done in a solution in which the ionic composition and concentration of the fluid outside of the bacteria is the same as that inside the bacteria. Once the structural support of the peptidoglycan is lost, the bacteria are unable to control their response to differences in the ionic composition between the bacterial interior and exterior. If the inner concentration is greater than the outer ionic concentration, water will flow into the bacterium in an attempt to achieve an ionic balance. The increased volume can be so severe that the bacteria will burst. Conversely, if the inner ionic concentration is less than the exterior, water will exit the bacterium, in an attempt to dilute the surroundings. The bacteria can shrivel to the point of death.

Preservation of ionic balance is required to ensure that bacteria will not be killed during their **transformation** into either the protoplast or the spheroplast form. Living protoplasts and spheroplasts are valuable research tools. The membrane balls that are the protoplasts or spheroplasts can be induced to fuse more easily with similar structures as well as with eukaryotic cells. This facilitates the transfer of genetic material between the two cells. As well, the sequential manufacture of spheroplasts and protoplasts in Gram-negative bacteria allows for the selective release of the contents of the **periplasm.** This approach has been popular in the identification of the components of the periplasm, and in the localization of proteins to one or the other of the Gram-negative membranes. For example, if a certain protein is present in a spheroplast population, but is absent from a protoplast population, then the protein is located within the outer membrane.

Some Useful Applications

Protoplasts and spheroplasts are valuable research tools and can be used in a wide range of applications:

Protoplasts

- Study of membrane biology, particularly in analysing uptake and transport processes Somaclonal variation in plant tissues.
- DNA transformation, especially in the creation of genetically modified organisms.
- Plant breeding, specifically in the generation of somatic hybrids in tissue culture.
- In Fluorescence Activated Cell Sorting (FACS), specifically in isolating certain cell types for further investigation

Spheroplasts

- Antibiotic recovery, particularly for antibiotics that inhibit cell wall biosynthesis.
- For studying the function of bacterial ion channels using the “patch clamp” technique.
- Transfection of animal cells.
- Facilitation of cell lysis