General introduction

The world of living things can be divided in the domains of procarya (bacteria-eubacteria and archaea, virus), and eucarya.. Procarya includes all life forms with cells without a genuine nucleus. They are bacteria and viruses. The word bacteria do not means neither parasite nor pathogen. Etymologically it means rod form. Out of millions species, there are just around 200 found pathogens or harmful to human. Many are beneficial but the few hurtful ones have kill more than weapon throughout history, and are continuing their destruction in the modern era despite civic sanitation, water purification, immunization, and antibiotics that have dramatically reduced the overall morbidity and the mortality due to disease. We need to surround the bacteria, master the few we know in other to improve our life: out of the microbes, bacteria, especially the harmful ones are a great constraint for us. It is estimated that eubacterial species on Earth number in the hundreds of thousands, of which only about 5500 have been discovered and described in detail.

Prokaryotic and Eukaryotic Microorganisms

Table 1: Characteristics of Prokaryotic (Eubacteria) and Eukaryotic (Fungi, Protozoans) microorganisms.

Characteristic	Prokaryotes (bacteria)	Eukaryotes (fungi, protozoans)
Nuclear structure	Circular DNA molecule not covered with proteins	Complex of DNA and basic proteins
Localization of nuclear structure	Dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent	In nucleus surrounded by nuclear membrane
DNA	Nucleoid and plasmids	In nucleus and in mitochondria
Cytoplasm	No mitochondria and no endoplasmic reticulum, 70S ribosomes	Mitochondria and endoplasmic reticulum, 80S ribosomes
Cell wall	Usually rigid wall with murein layer; exception: mycoplasmas	Present only in fungi: glucans, mannans, chitin, [illegible] cellulose
Reproduction	Asexual, by binary transverse fission	In most cases sexual, possibly asexual

choline, or lecithin, which is characteristic of higher organisms. Other bacteria produce glycolipids, such as monogalactosyl diglyceride. The membrane lipids from archaea are quite different from those in bacteria and eukarya. Their hydrocarbon chains are based on isoprenoid units and these are linked to the glycerol backbone in an ether, rather than ester, linkage. In some archaea, a glycerol backbone and head group are attached to both ends of a pair of isoprenoid units.

Sterols, such as cholesterol in mammalian cells or ergosterol in fungi, are invariant features of membranes in eukaryal cells, in which they appear to stiffen the membrane by increasing the degree of order of the hydrocarbon chains. Sterols are not com-monly found in bacteria and archaea, except for the cell wall-less Mycoplasma. Very complex lipids, including the very long, branched mycolic acids, are common in Mycobacterium but occur in a very thick and rigid outer layer rather than in the cytoplasmic membrane.

The cell membrane is involved in energy generation (mainly respiration, photosynthesis- A completely different system for conversion of light into metabolic energy is present in *Halobacterium salinarum*, an extremely halophilic archaeon that thrives in very saline environments such as the Dead Sea or brine evaporation ponds. These bacteria produce patches of membrane that are densely packed with the membrane protein, bacteriorhodopsin, having seven transmembrane helices and covalently bound retinal as in the visual pigment in mammalian retina., coupled processes- Some bacteria couple the transport and metabolism of their energy source directly to the production of the pmf. An example of this very simple, but not very energy-rich, process is malo-lactate fermentation in *Leuconostoc*. The substrate malate is transported into the cell and converted to lactate, which leaves the cell in exchange for a new molecule of malate.), membrane transport, export of surface molecules, and cell growth (movement, division and signal transduction).

I.2.4. Cell wall

The tasks of the complex bacterial cell wall are to protect the protoplasts from external noxae, to withstand and maintain the osmotic pressure gradient between the cell interior and the extracellular environment (with internal pressures as high as 500–2000 kPa, -2-25 atm), to give the cell its outer form and to facilitate communication with its surroundings. Eubacteria as a rule reinforce their cell envelope by an exoskeleton made of peptidoglycan (murein), a cross-linked biopolymer that is extremely well suited to endow the cell with sufficient strength. In order to achieve this, the murein forms a closed bag-shaped structure, called sacculus, completely wrapping up the cell. It is made up of polysaccharide (glycan strands) chains crosslinked by peptides **(Figure 2 & 3)**. Importantly, the murein sacculus not only stabilizes the cytoplasmic membrane but also maintains the specific shape of the bacterium. Morphogenesis of bacteria can therefore be studied by analyzing the metabolism of a single macromolecule—the murein sacculus. On the basis of a special staining procedure bacteria can be subdivided in essentially two groups, the Gram-positive and the Gram-negative bacteria.

a) The cell wall of Gram-positive bacteria

The murein sacculus may consist of as many as 40 layers (15–80 nm thick) and account for as much as 30 % of the dry mass of the cell wall. The membrane lipoteichoic acids are anchored in the cytoplasmic membrane, whereas the cell wall teichoic acids are covalently coupled to the murein (**Fig 2**). The physiological role of the teichoic acids is possibly to regulate the activity of the autolysins that steer growth and transverse fission processes in the cell. Within the macroorganism, teichoic acids can activate the alternative complement pathway and stimulate macrophages to secrete cytokines. Examples of cell wall-associated proteins are protein A, the clumping factor, and the fibronectin-binding protein of *Staphylococcus aureus* or the M protein of *Streptococcus pyogenes*. Cell wall-associated proteins frequently function as **pathogenicity determinants** (specific adherence; phagocyte protection).

I.2.5. Capsule and slim layers (all called glycocalyx)

Many bacterial cells secrete some extracellular material in the form of a capsule or a slime layer that surround the cell wall. A slim layer can be easily wash off because it is loosely associated with the bacterium, whereas a capsule is tightly attached to the bacterium. Capsules are usually made of polysaccharides, but some, like the one of *Bacillus anthracis* is made of polyglutamic acids, precisely the unusual aminino acid O-glutamic acid. From their structure, most capsules are hydrophilic, thus preventing bacterial desiccation. They can also protect bacteria from being phagocytise, by improving slippery or not being recognised as a stranger element. This gives a strong correlation between the possession of a capsule and virulence of many organisms. In example, the presence of capsule in *Streptococcus pneumoniae* (*Diplococcus pneumoniae*) determines his ability to cause pneumonia. Mutant strains of *S. pneumoniae* that cannot form capsules also loss the ability to cause disease because they are easily phagocytised. It arrives the same for *B. anthracis*. The polysaccharide capsule of *Klebsiella pneumonia* also prevents phagocytosis and allows the bacterium to adhere to and colonize the respiratory tract.

The bacteria of a single species can be classified in different capsular serovars (or serotypes) based on the fine chemical structure of capsular polysaccharide.

Special case of bacterial biofilm

In nature, microorganisms may exist as single cells that float or swim independently in a liquid, or they may attach to each other and/or some usually solid surface. This latter mode of behaviour is called a biofilm, a complex aggregation of microbes not to confuse with bacterial arrangement. A bacterial biofilm is a structured community of bacterial cells embedded in a self-produce d polymer matrix and attached to either an inert surface or living tissue. Such films can develop considerable thickness (mm). Biofouling is the damage caused to a surface by microorganisms attached to a surface. The bacteria are able to organise themselves into a consortia: Spatial grouping of bacterial cells within a biofilm in which different species are physiologically coordinated with each other, so as to produce phenomenally efficient chemical transformations. The bacteria located deep within a biofilm structure are called sessile, and can be isolated from immune system cells, antibodies and antibiotics.

The polymers they secrete are generally glycosides, from which the term glycocalyx (glycoside cup) for the matrix is derived. The slime covering a rock in a lake is a biofilm. In the case of bacterial biofilm, extracellular polysaccharide material enables many bacteria into a film and serve many functions.

EPS (exopolysaccharides) and glycocalyx are terms used to describe the polysaccharide produced by bacterial cells. EPS refers to one of the major components of biofilms, and glycocalyx refers to the polysaccharide matrix surrounding individual cells. EPS has an important role in biofilm structure and function and has a complex physical and chemical nature. Its functions are mostly protective in nature and this is one of the benefits for bacteria in the sessile state. Because the glycocalyx is the outermost component of bacterial cells, this layer mediates virtually all bacterial associations with surfaces and other cells: it dictates location, juxtaposition, and the eventual success in the ecosystem.

*Streptococcus mutans*_split the sucrose in food and use one of the sugars to build a capsule, which sticks tightly to the tooth. The bacteria that are trapped in the common capsule use the other sugar as food and produce an acidic waste (lactic acid) that attack the tooth enamel and gives dental carries. (comment need of brush). *Pseudomonas aeruginosa* use to produce a thick capsular polymer of alginic acid that contribute to the difficulty of eradicating the bacterium when it colonises the lungs with cystic fibrosis.some other bacteria can secrete fibres of cellulose or long chemically complex tubular sheath that can become encrusted with iron or manganese oxides.

Examples:- cellulose from the genus *Zoogloea* enmeshes the bacteria into a flock (groupe) that floats on the surface of liquid.

- Rod-shaped bacteria like such as *Sphaerotilus* sp secrete complex tubular sheets.

Chapter Two: Nutrition growth and cultivation in bacteria

Introduction

The ability of bacteria to thrive in specific habitats is a function of both nutritional and physical factors. Each type of bacteria has specific growth requirements, which may be decided for example by an organism's ability to metabolize only one kind of carbohydrate or to tolerate only certain pH levels and temperatures. Thus, the relationship between bacteria and the environment, as well as between bacteria and host, is a reflection of their specific growth requirements.

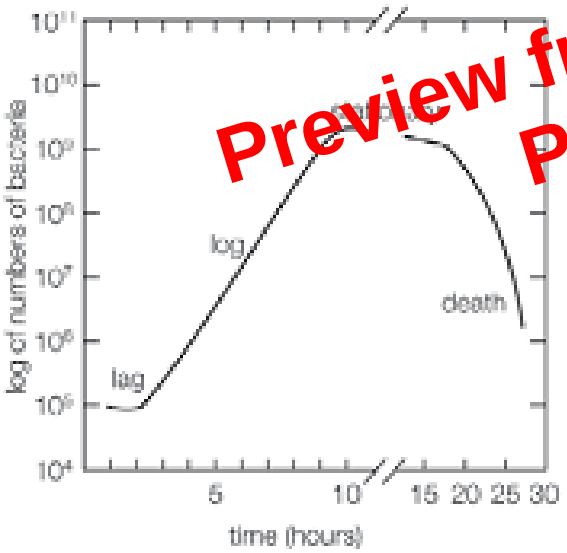
II.1. Growth of bacterial populations

Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth. The time required for the formation of a generation, the generation time (G), can be calculated from the following formula:

$$G = \frac{t}{n} = \frac{t}{3.3 \log b/B}$$

In the formula, B is the number of bacteria present at the start of the observation, b is the number present after the time period t, and n is the number of generations. The relationship shows that the mean generation time is constant and that the rate at which the number of bacteria increases is proportional to the number of bacteria at any given time. This relationship is valid only during the period when the population is increasing in an exponential manner, called the log phase of growth. For this reason, graphs that show the growth of bacterial cultures are plotted as the logarithm of the number of cells.

The generation time, which varies among bacteria, is controlled by many environmental conditions and by the nature of the bacterial species. For example, *Clostridium perfringens*, one of the fastest-growing bacteria, has an optimum generation time of about 10 minutes; *Escherichia coli* can double every 20 min; and the slow-growing *Mycobacterium tuberculosis* has a generation time in the range of 12 to 16 h. The growth rate increases up to a maximum when the medium provides a better energy source and more of the biosynthetic intermediates that the cell would otherwise have to make for itself.



When bacteria are placed in a medium that provides all of the nutrients that are necessary for their growth, the population exhibits four phases of growth that are representative of a typical bacterial growth curve.

(1). Upon inoculation into the new medium, bacteria do not immediately reproduce, and the population size remains constant. During this period, called the lag phase, the cells are metabolically active and increase only in cell size. They are also synthesizing the enzymes and factors needed for cell division and population growth under their new environmental conditions.

(2). The population then enters the log phase, in which cell numbers increase in a logarithmic fashion, and each cell generation occurs in the same time interval as the preceding ones, resulting in a balanced increase in the constituents of each cell. The log phase continues until nutrients are depleted or toxic products accumulate, at which time the cell growth rate slows, and some cells may begin to die. Under optimum conditions, the maximum population for some bacterial species at the end of the log phase can reach a density of 10 to 30 billion cells per millilitre.

(3). The log phase of bacterial growth is followed by the stationary phase, in which the size of a population of bacteria remains constant, even though some cells continue to divide and others begin to die.

Chapter Three: Classification of bacteria

Introduction: history

Scientists observe each organism, noting its characteristics. Organisms that have similar characteristics are presumed to have a natural relationship and therefore are placed in the same group. Classification tries to show this natural relationship. Taxonomy has three components:

- Classification. The arrangement of organisms into groups based on similar characteristics, evolutionary similarity or common ancestry. These groups are also called *taxa*.
- Nomenclature. The name given to each organism. Each name must be unique and should depict the dominant characteristic of the organism.
- Identification. The process of observing and classifying organisms into a standard group that is recognized throughout the biological community.

Taxonomy is a subset of systemics. Systemics is the study of organisms in order to place organisms having similar characteristics into the same group. Using techniques from other sciences such as biochemistry, ecology, epidemiology, molecular biology, morphology, and physiology, biologists are able to identify characteristics of an organism.

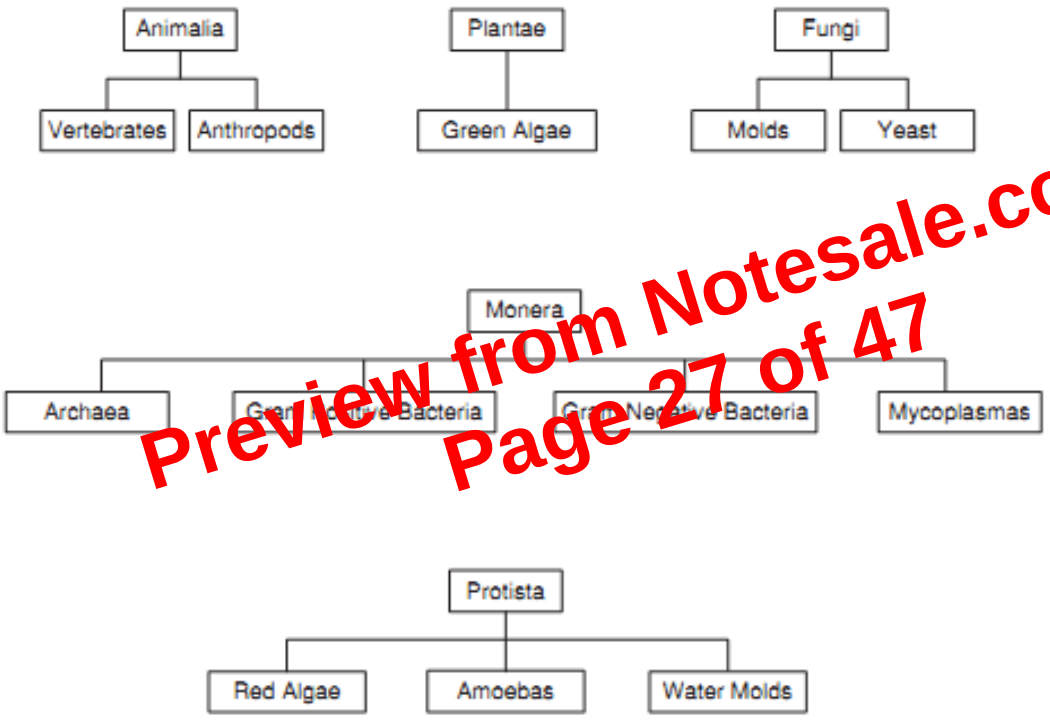


Figure 9: Whitteker's five-kingdom taxonomy.

Scientists widely accepted Whitteker's taxonomy until 1977 when Carl Woese, in collaboration with Ralph S. Wolfe, proposed a new six-kingdom taxonomy. This came about with the discovery of archaea,

Woese's six-kingdom taxonomy consists of:

- Eubacteria (has rigid cell wall);
- Archaebacteria (anaerobes that live in swamps, marshes, and in the intestines of mammals);
- Protista (unicellular eukaryotes and algae);
- Fungi (multicellular forms and single-cell yeasts);
- Plantae;
- Animalia.

Woese determined that Archaebacteria and Eubacteria are two groups by studying the rRNA sequences in prokaryotic cells.

Today organisms are grouped into three categories called domains that are represented as Bacteria, Archaea, and Eukarya. The domains are placed above the phylum and kingdom levels. The term

among organisms have proved a reliable means of assigning a strain to an existing species or establishing the basis for a new species. Nevertheless it is curious to see that assigning bacterial genera to higher taxonomic levels—families, orders, classes, and divisions (or phyla)—can be even more difficult than organizing species and strains within genera. This problem is well highlighted by the fact that organisms are sometimes moved from one category to another, and their official names are sometimes changed. For example, the bacterium that causes tularemia, a fever acquired by handling infected rabbits, was for many years called *Pasturella tularensis*. Its genus name was changed to *Francisella* after DNA hybridization studies revealed that hybridization between its DNA and that of *Pasteurella* species did not occur. It does, however, have a 78% match with the DNA of *Francisella novicida*. Efforts are being made to classify bacteria by evolutionary relationships, too, but these efforts are hampered by the incompleteness of the fossil record and by the limited information gleaned from what fossils have been found.

Some groups of bacteria, such as Rickettsiae and Chlamydiae, contain rather unusual organisms. These two groups are obligate intracellular parasites. Chlamydiae have an interesting and complex life cycle rather than dividing by binary fission, as do Rickettsiae and most other bacteria. Mycoplasmas lack cell walls and form colonies that look like eggs fried sunny-side up. They have sterols in their cell membranes that give them great flexibility of shape. Also interesting are the Ureaplasmas, also with unusual cell walls and/or cell membranes **(table 3.1)**.

table 3.1: Characteristics of typical Bacteria, Rickettsiae, Chlamydiae, Mycoplasmas, Ureplasmas and viruses

Characteristics of Typical Bacteria, Rickettsiae, Chlamydiae, Mycoplasmas, Ureaplasmas, and Viruses

Characteristic	Typical Bacteria	Rickettsiae	Chlamydiae	Mycoplasmas	Ureaplasmas	Viruses
Cell wall	Yes	Yes	Yes	No	Sometimes	No
Grow only in cells	No	Yes	Yes	No	No	Yes
Require sterols	No	No	No	Sometimes	Yes	No
Contain DNA and RNA	Yes	Yes	Yes	Yes	Yes	No
Have metabolic system	Yes	Yes	Yes	Yes	Yes	No

Bacteria can be systematically separated into groups on the basis of morphological or shared (common) evolutionary relationships. Today the classification of bacteria is grounded primarily in genetics. However, whereas genetic classification is valuable in understanding evolutionary relationships, morphological and biochemical features continue to play important roles in the functional identification and grouping of bacteria, particularly for the purposes of medicine.

III.1.1. Phenetic classification

It groups organisms together based on the mutual similarity of their phenotypic characteristics. Although phenetic studies can reveal possible evolutionary relationships, they are not dependent on phylogenetic analysis. A good classification should bring order to biological diversity and may even clarify the function of a morphological structure.

III.1.2. Numerical taxonomy

The development of computers has made possible the quantitative approach known as **numerical taxonomy**. It is the grouping by numerical methods of taxonomic units into taxa on the basis of their character states. Information about the properties of organisms is converted into a form suitable for numerical analysis and then compared by means of a computer. The results of numerical taxonomic analysis are often summarized with a treelike diagram called a **dendrogram.**

III.1.3. Phylogenetic / phyletic classification

These are systems based on evolutionary relationships rather than general resemblance (the term **phylogeny** refers to the evolutionary development of a species). This has proven difficult for procaryotes and other microorganisms, primarily because of the lack of a good fossil record. The direct comparison of genetic material and gene products such as RNA and proteins overcomes many of these problems.

IV.2. Penetration into host defences

Although some pathogens can cause damage on the surface of tissues, most must penetrate tissues to cause disease. This ability is related to many factors.

IV.2.1. Capsule

The chemical nature of the **capsule** appears to prevent the phagocytic cell from adhering to the bacterium, and so increasing virulence. However, the human body can produce antibodies against the capsule, and when these antibodies are present on the capsule surface, the encapsulated bacteria are easily destroyed by phagocytosis. One bacterium that owes its virulence to the presence of a polysaccharide capsule is *Streptococcus pneumoniae*, *-Haemophilus influenzae*, *Bacillus anthracis*, *Yersinia pestis*, *Klebsiella pneumoniae*,etc. Also have virulence determine by capsule.

IV.2.2. Cell walls

The **cell walls** of certain bacteria contain chemical substances that contribute to virulence. For example, *Streptococus pyogenes* produces a heat - resistant and acid-resistant protein called M protein found on the cell surface and fimbriae. The M protein mediates attachment of the bacterium to epithelial cells of the host and helps the bacterium resist phagocytosis by white blood cells. *Neisseria gonorrhoeae* can grow inside human epithelial cells and leukocytes. These bacteria use fimbriae and an outer membrane protein called Opa to attach to host cells. The host cells then take in the bacteria. The waxy lipid (mycolic acid) that makes up the cell wall of *Mycobacterium tuberculosis* also increases virulence by resisting digestion by phagocytes, and can even multiply inside phagocytes.

IV.2.3. Enzymes

The virulence of some bacteria is aided by the production of extracellular **enzymes** (exoenzymes) and related substances. These chemicals can digest materials between cells and form or digest blood clots, among other functions.

Coagulases are bacterial enzymes that coagulate (clot) the fibrinogen in blood changing it to fibrin. Fibrinogen, a plasma protein produced by the liver, is converted by coagulases into fibrin, the threads that form a blood clot. The fibrin clot may protect the bacterium from phagocytosis and isolate it from other defenses of the host. Coagulases are produced by some members of the genus *Staphylococcus*.

Bacterial kinases are bacterial enzymes that break down fibrin and thus digest clots formed by the body to isolate the infection. One of the better- known kinases are fibrinolysin (or streptokinase), and staphylokinase.

Hyaluronidase is another enzyme secreted by certain bacteria, such as streptococci. It hydrolyzes hyaluronic acid, a type of polysaccharide that holds together certain cells of the body, particularly cell s in connective tissue. This digesting action is thought to be involved in the tissue blackening of infected wounds and to help the microorganism spread from its initial site of infection. Hyaluronidase is also produced by some clostridia that cause gas gangrene.

Other enzymes are collagenase, produced by several species of *Clostridium*; IgA proteases, that can destroy these antibodies. *N. gonorrhoeae* has this ability, as do *N. meningitidis* and other microbes that infect the central nervous system.

IV.2.4. Antigenic variation

Some pathogens can alter their surface antigens, by a process called **antigenic variation**. Thus, by the time the body mounts an immune response against a pathogen, the pathogen has already altered its antigens and is unaffected by the antibodies. *N. gonorrhoeae* has several copies of the Opa-encoding gene, resulting in cells with different antigens and in cells that express different antigens over time.

IV.2.5. Penetration into the host cell cytoskeleton

As previously noted, microbes attach to host cells by adhesins. The interaction triggers signals in the host cell that activate factors that can result in the entrance of some bacteria.