

CHAPTER 01: INTRODUCTION to SYSTEMATICS

1. Microbial cell

Microbiology can be defined as the study of organisms that are too small to be clearly visible to the naked eye. This will include all organisms with a diameter of less than approximately 1mm. The development of electron microscopy showed that there was a major dichotomy between the various groups relating to the internal structure and organization of cells. Two very different types of cells were discovered, the small, relatively simple prokaryotic cell, and the more complex eukaryotic cell which is usually considerably larger.

2. Prokaryotic cells

The vast majority of prokaryotic organisms are unicellular, although multicellular bacteria forming filaments are also found (Fig. 01). Prokaryotic cells (Greek *Pro*, before, and *Karyon*, nut or kernel; organisms with a primordial nucleus) are surrounded by a cell membrane, a thin flexible sheet composed of protein and lipid. This has an important function in regulating the molecules which can pass into and out of the cell.

In the great majority of cases, there is a rigid cell wall outside the membrane. This cell wall has a chemistry which is unique to the prokaryotic cell, and contains a number of compounds which are not found in the eucaryotes (peptidoglycan or murein). A few groups such as *Mycoplasma* do not possess a cell wall.

In the prokaryotes, there is no discrete nucleus surrounded by a nuclear membrane, and the deoxyribonucleic acid (DNA) is not associated with the basic proteins known as histones. The DNA consists of covalently closed circles.

There are no organelles such as mitochondria present within the cell. Flagella are present in the case of motile cells; the position of these varies from species to species and may be used diagnostically.

Certain species may contain spores which are highly resistant to heat and a variety of chemicals. A number of species are capable of photosynthesis, that is they can convert solar energy into chemical energy.

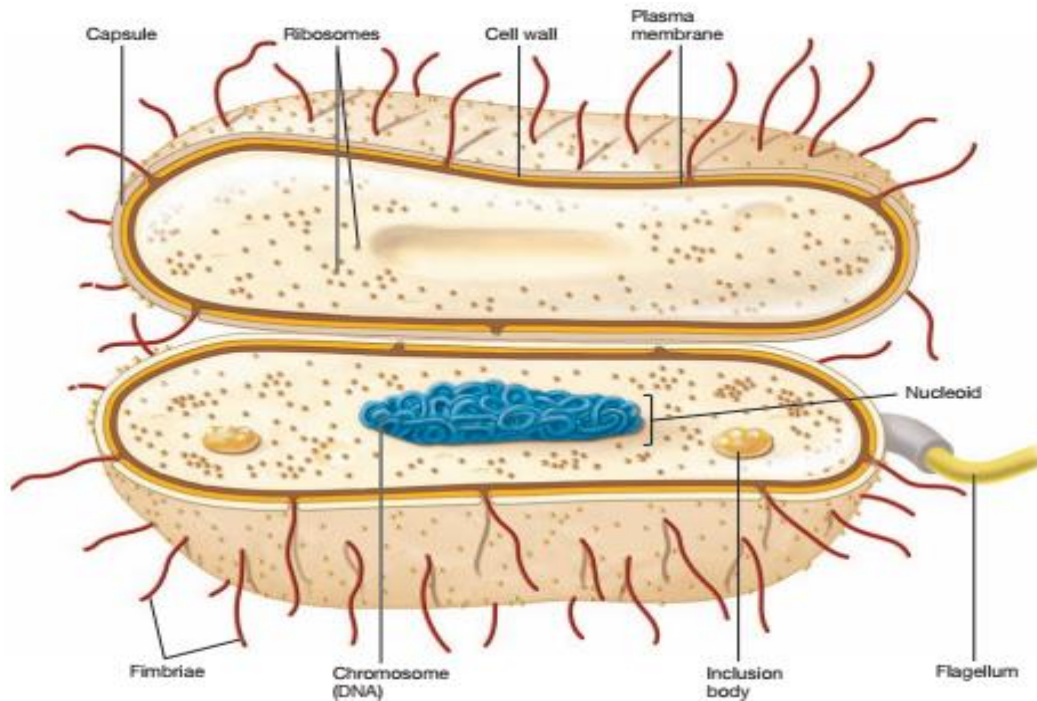


Figure 01: Morphology of a prokaryotic cell

There is also a considerable difference in the ribosome structure of prokaryotic and eukaryotic cells. Ribosomes are the structures upon which proteins are synthesized. Prokaryotic cells possess ribosomes which are 70 S in size, whereas eucaryotes have 80 S ribosomes.

2.1. Bacteria

They are prokaryotes that are usually single-celled organisms. Most have cell walls that contain the structural molecule peptidoglycan. They are abundant in soil, water, and air, and are major inhabitants of our skin, mouth, and intestines. Some bacteria live in environments that have extreme temperatures, pH, or salinity. Although some bacteria cause disease, many more play beneficial roles such as cycling elements in the biosphere, breaking down dead plant and animal material, and producing vitamins. *Cyanobacteria* (once called blue-green algae) produce significant amounts of oxygen through the process of photosynthesis.

2.2. Archaea

They are prokaryotes that are distinguished from *Bacteria* by many features, most notably their unique ribosomal RNA sequences. They lack peptidoglycan in their cell walls and have unique membrane lipids. Some have unusual metabolic characteristics, such as the methanogens,

which generate methane gas. Many *Archaea* are found in extreme environments, including those with high temperatures (thermophiles) and high concentrations of salt (extreme halophiles). Pathogenic *Archaea* have not yet been identified.

3. Taxonomy definition

Microbiologists are faced with the daunting task of understanding the diversity of life forms that cannot be seen with the naked eye but can live seemingly anywhere on Earth. One of the first tools needed to survey this level of diversity is a reliable classification system.

Taxonomy (Greek *taxis*, arrangement or order, and *nomos*, law, or *nemein*, to distribute or govern) is defined as the science of biological classification. The term **systematics** is often used for **taxonomy**.

Systematics is, in more general terms, the scientific study of organisms with the ultimate object of characterizing and arranging them in an orderly manner. Any study of the nature of organisms, when the knowledge gained is used in taxonomy, is a part of systematics. Thus, systematics encompasses disciplines such as morphology, ecology, epidemiology, biochemistry, molecular biology, and physiology.

In a broader sense, the taxonomy consists of three separate but interrelated parts: classification, nomenclature, and identification. These three areas are interrelated and play a vital role in keeping a dynamic inventory of the extensive array of living things.

- **Classification** scheme is selected, it is used to arrange organisms into groups called **taxa** (s., **taxon**) based on mutual similarity.

- **Nomenclature** is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.

- **Identification** is the practical side of taxonomy, the process of determining if a particular isolate belongs to a recognized taxon (the matching of an unknown organism with a known species).

4. Taxonomy importance

Taxonomy is important for several reasons:

First, it allows us to organize the enormous amount of knowledge we have about organisms, because all members of a given group share many characteristics. It's, in a sense, like a giant filing

system or a library catalog that provides easy access to information. The more precise the classification, the more useful and informative it is.

Second, taxonomy allows us to make predictions and hypotheses for future research based on knowledge of similar organisms. If a related organism has certain properties, the one being studied may have the same characteristics.

Third, taxonomy divides microorganisms into meaningful, useful groups with precise names so that microbiologists can study and communicate effectively. Just as effective written communication is not possible without adequate vocabulary, correct spelling, and good grammar, microbiology is not possible without taxonomy.

Fourth, taxonomy is essential for the accurate identification of microorganisms.

5. Basic taxonomic group in microbial taxonomy

The **species** is the basic taxonomic group in microbial taxonomy. Taxonomists working with higher organisms define the term species differently than do microbiologists. Species of higher organisms are groups of interbreeding or potentially interbreeding natural populations that are reproductively isolated from other groups. This is a satisfactory definition for organisms capable of sexual reproduction but fails with many microorganisms because they do not reproduce sexually. The division between species at the plant and animal levels is generally relatively easy on the basis of their morphology (appearance), although problems can arise in distinguishing between the simpler algae and protozoa. This however is not true with bacteria as the range of distinguishable shapes and sizes is too small.

A **prokaryotic species** is a collection of strains that share many stable properties and differ significantly from other groups of strains. According to some bacterial taxonomist, a species (genomospecies) is a collection of strains that have a similar G + C composition and 70% or greater similarity as judged by DNA hybridization experiments. A **species** should be also phenotypically distinguishable from other similar species

A **strain** is a population of organisms that is distinguishable from, at least, some other populations within a particular taxonomic category. Strains within a species differ slightly from one another. It consists of the descendants of a single, pure microbial culture. Strains of the same

species are distinguished by following the specific epithet with a number, a letter, or a name. For example, *E.coli* strain O157 :H7 is responsible for the diarrhea associated with Hamburger disease.

There are a number of different ways in which strains within a species may be described:

- **Biovars** (or **biotype**): have special biochemical or physiological properties;
- **Morphovar** (or **morphotype**): have special morphologic features;
- **Serovar** (or **serotype**): have distinctive antigenic properties;
- **Pathovars** (or **pathotype**): have pathogenic properties for certain hosts;
- **Phagovar** (or **phage type**): have the ability to be lysed by certain bacteriophages;
- **Zymovars** (or **zymotype**): have differences in enzyme isotypes;
- **Antibiotics**: have differences in sensitivity to antibiotics.

Type strain, for each species, one strain is designated as the **type strain**. It is usually one of the first strains studied and often is more fully characterized than other strains; however, it does not have to be the most representative member. The type strain for the species is called the **type species** and is the nomenclatural type or the holder of the species name. A **nomenclatural type** is a device to ensure permanence of names when taxonomic rearrangements take place. When nomenclature revisions occur, the type species must remain within the genus of which it is the nomenclatural type.

6. Taxonomic ranks

The classification of microbes involves placing them within hierarchical taxonomic levels. Microbes in each level or rank share a common set of specific features. The ranks are arranged in a nonoverlapping hierarchy so that each level includes not only the traits that define the rank above it but also a new set of more restrictive traits (Fig. 02).

In taxonomy, the **strains** are grouped into individual species which are then grouped into progressively higher series of categories. In order, each **species** is assigned to a genus, the next rank in the taxonomic hierarchy. A **genus** is a well-defined group of one or more species that is clearly separate from other genera or a genus. Category containing a single series or a monophyletic group of species, which is separated from other genera by a decided gap. The next rank in the taxonomic classification is **family** which is a taxonomic category containing one or more related genera and separated from other related families by important and characteristic differences. Next

group of taxonomic classification is **order** which may include *super orders* or *suborders*. **Class** is the subdivision of a phyla. A class is the basic category of the class group which may include *super class* or *infra class*. The next category of taxonomic classification is **phylum (division)** which is divided into *super phylum* or *sub phylum*. The **kingdom** is the highest taxonomic category. All animals are included in animal kingdom and all plants are included in the plant kingdom. The **domain** is a collection of similar kingdoms. It is a relatively new taxonomic category that reflects the characteristic of the cells that make up the organism.

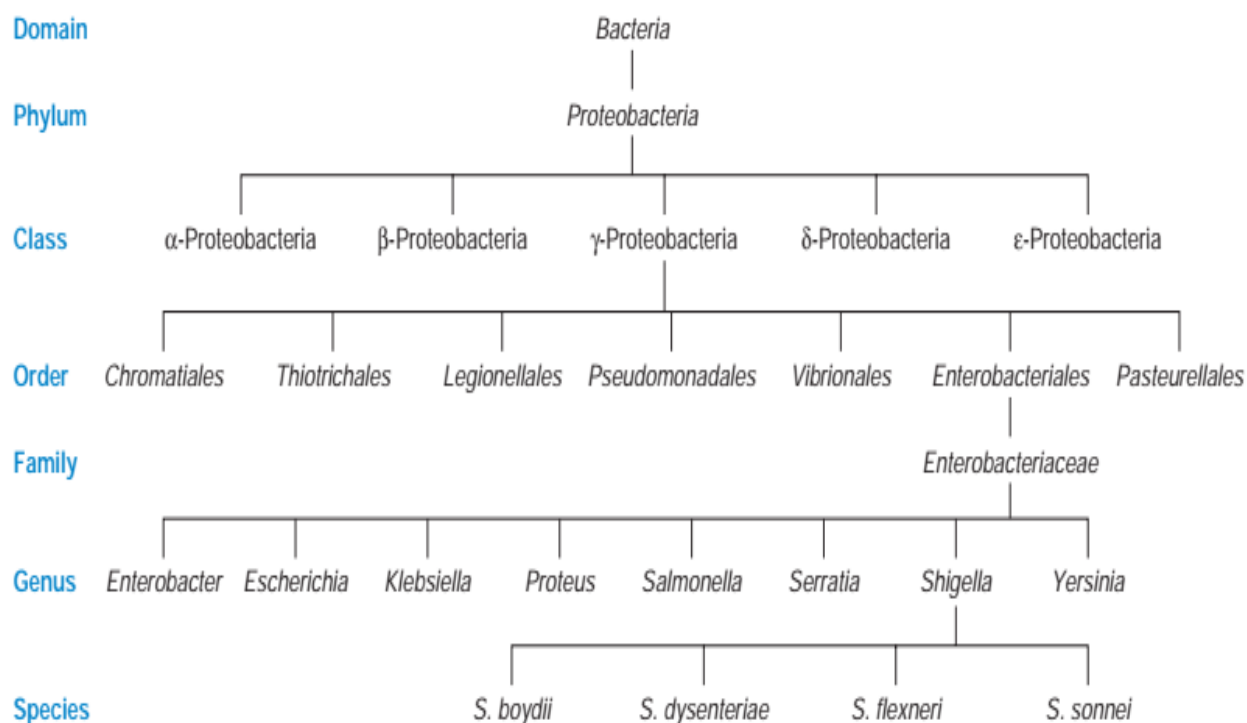


Figure 02: Hierarchical Arrangement in Taxonomy.

7. Systematic different parts

7.1. Classification

A classification system based on the scheme of assigning individuals to group and assigning these to progressively more inclusive and broader groups is called a *hierarchical scheme of classification* (Fig. 02).

7.1.1. Classification history

The classification of microorganisms began in 1674 with the invention of light microscope and today is a discipline based on increasingly complex criteria. The earliest schemes assigned

microorganisms to one or the other of the two major categories of living things plants and animals i.e two kingdom **Plantae** and **Animalia**. In the nineteenth century, scientists began to realize that the assignments of certain members viz. Protozoa, Algae, Fungi and Bacteria into these two kingdoms is very artificial for example: microorganism are neither plants nor animals. Nearly every group has representatives with plant like properties and others with animal like properties (if it ran around, it was an animal, if it was green and didn't, it was a plant!).

a. Thus, the German biologist Ernst Haeckel in 1866 proposed a three kingdoms concept Plants, Animals and microorganism (the **Protista**). He proposed that the *bacteria, algae, fungi* and *protozoa* that lacked tissue differentiation be removed from the plant and animal kingdoms and be separated into third kingdom called **Protista**.

b. In the 20th century, several systems of classification were proposed but the one that is most widely accepted was given by the American taxonomist Robert H. Whittaker (1969). This system places all living things (except the viruses) into **five kingdoms** based on cellular organization and nutritional patterns (i) the procaryotae or **Monera** (ii) the **Protista** (iii) the **Myceteae** or fungi (iv) the **Plantae** (v) the **Animalia** (Tab. 01).

Table 01: Whittaker's five kingdoms concept.

Property	Plantae	Animalia	Protista	Fungi	Monera
Cell type	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Prokaryotic
Cell organization	Mostly multi-cellular	Mostly multi-cellular	Mostly uni-cellular	Multicellular and unicellular	Mostly unicellular
Cell wall	Present	Absent	Present in some, absent in others	Present	Present in most
Nutritional class	Phototrophic	Heterotrophic	Heterotrophic and Phototrophic	Heterotrophic	Phototrophic, heterotrophic or Chemoautotrophic
Mode of nutrition	Mostly absorptive	Mostly ingestive	Absorptive or ingestive	Absorptive	Absorptive
Motility	Mostly nonmotile	Mostly motile	Motile or nonmotile	Non motile	Motile or non motile
Example	Algae, mosses, ferns, all other plants	Invertebrates, vertebrates	Protozoans, slime molds, some algae	Molds, yeasts, mushrooms, rusts and smuts	Eubacteria, Archaeobacteria

c. The five-kingdom system is not accepted by many biologists. For example, the brown algae are probably not closely related to plants even though the five-kingdom system places them in the plantae. Because of these problems with five kingdom system, **six-kingdom system** was the simplest option where the kingdom **Monera** (or prokaryote) divided into two kingdoms viz. **Eubacteria** and **Archaeobacteria**.

d. By the 1970's, molecular biologists realized that prokaryotic consist of two different and unrelated groups. They are as distantly related to each other as they are to eukaryotes. To accommodate this new information three microbiologists C. Woese, O. Kandler and M.L. Wheelis introduced a new classification scheme in 1990. They proposed that all organisms be divided into three major groups or super kingdoms called **domains**: the **Eukarya** (containing all eukaryotes), the **Bacteria** (containing most familiar prokaryotes), and the **Archaea** (originally called archaea bacteria and containing prokaryotes that live mostly in extreme environments) (Fig. 03).

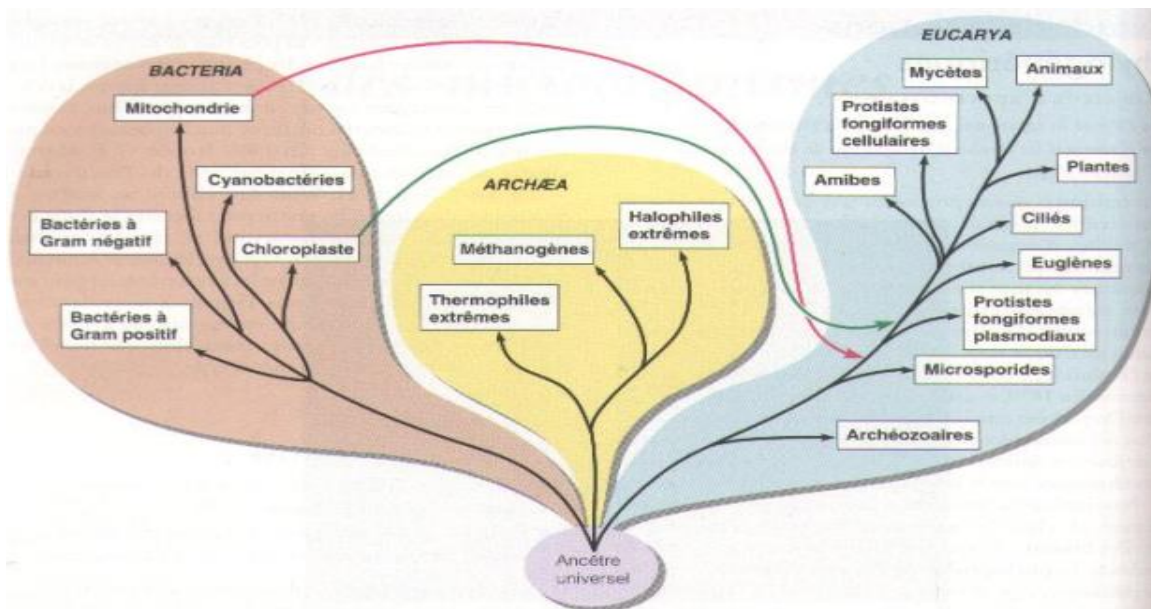


Figure 03: Three-domain system.

7.1.2. Classification types

There are two main types: artificial or natural.

7.1.2.1. Artificial classification

This classification groups organisms together based on overall similarity from artificially gathered phenetic, genetic, chemical, immunological, or ecological characters. It is based on a key

that collects together a set of bacteria sharing the same phenetic property: physiological or metabolic, easily recognizable by their presence (+) or absence (-). It is based on the prevalence of a few arbitrarily chosen traits.

This type of taxonomy is of great practical interest since it limits the scope of investigation a priori to only bacteria sharing the discriminating property. It arises from the fact that bacteriologists do not all have the same approach to microbiology (medical, agri-food, biotech, fundamental, etc.). Each field tends to use its own criteria for classification, which means that some bacteria may have different names. For example: *Erwinia hubicola* (plant saprophyte) = *Enterobacter agglomerans* (intestinal bacterium) or *Bacillus cereus* = *Bacillus thurengiensis*.

7.1.2.2. Natural classification

One of the oldest classification systems, it arranges organisms into groups whose members share many characteristics and reflects as much as possible the biological nature of organisms. The Swedish botanist Carl Von Linné (Carolus Linnaeus) developed the first natural classification, based largely on anatomical characteristics, in the middle of the eighteenth century.

When natural classification is applied to higher organisms, evolutionary relationships become apparent simply because the morphology of a given structure (e.g., wings) in a variety of organisms (ducks, songbirds, hawks) suggests how that structure might have been modified to adapt to specific environments or behaviors. However, the traditional taxonomic assignment of microbes was not necessarily rooted in evolutionary relatedness. For instance, bacterial pathogens and microbes of industrial importance were historically given names that described the diseases they cause or the processes they perform (e.g., *Vibrio cholerae*, *Clostridium tetani*, and *Lactococcus lactis*).

7.2. Nomenclature

In the eighteenth century (1735), a Swedish biologist named Carolus Linnaeus devised a widely accepted scheme and he also laid down the basic rules for taxonomic categories or taxa and gave the **binomial system of nomenclature** i.e. naming of an organism by two names, *genus* and *species*. The name of the organism starts with the *generic* (genus) name that is always capitalized, which is followed by the uncapitalized *species* name or *specific epithet* that begins with small letter. Both names should be written in italics or underlined if italics are not available ex. *Eschericia coli*. Scientific names are in Latin because it was the language traditionally used by scientists. Carolus

Linnaeus (1707-1788), called as 'Father of Taxonomy', classified the organisms according to his own system of classification (Binomial system).

According to the *Bacteriological Code*, every scientific name must be a Latin word (the term designating the genus may, however, be borrowed from Greek) or a word Latinized by the addition of an appropriate suffix. For example, the suffixes used for the terms designating an order and family are *-ales* and *-aceae*, respectively (Fig. 02).

The species name is stable; the oldest epithet for a particular organism takes precedence and must be used. In contrast, a generic name can change if the organism is assigned to another genus because of new information. For example, some members of the genus *Streptococcus* were placed into two new genera, *Enterococcus* and *Lactococcus*, based on rRNA analysis and other characteristics. Thus, *Streptococcus faecalis* is now *Enterococcus faecalis*. Often the name will be shortened by abbreviating the genus name with a single capital letter, for example *E. coli*. When two bacteria begin with the same letter, to avoid confusion, it is preferable to abbreviate each genus by two or three letters: *Acinetobacter* (*Aci*) and *Actinomyces* (*Act*).

A number of species have been named in honor of a scientist who originally discovered the microbe or who had made outstanding contribution to the field, name a species habitat or describe the organism. For example, *Escherichia coli*, the generic name *Escherichia* is named after Theodor Escherich, a German bacteriologist who first described the bacterium, and specific name *coli* refers to the colon which is appropriate because this organism is an enteric resident of humans. Also, *Staphylococcus aureus* (found commonly on human skin), *Staphylo-* describes the clustered arrangement of cells, *-coccus* indicates that they are shaped like spheres. The specific epithet, *aureus*, means “golden” in Latin; a large number of colonies of this bacterium are of this color. In the media, the Latin scientific names of microbes are not very often used; thus, we will speak of golden staphylococcus instead of *Staphylococcus aureus*, the tetanus bacillus instead of *Clostridium tetani*, gonococcus instead of *Neisseria gonorrhoeae* or Koch's bacillus instead of *Mycobacterium tuberculosis*. The vernacular name of the microbes is then given.

A new prokaryotic species cannot be recognized until it has been published in the *International Journal of Systematic and Evolutionary Microbiology*; until that time, the new species name will appear in quotation marks. *Bergey's Manual of Systematic Bacteriology* contains the currently accepted system of prokaryotic taxonomy.

7.3. Identification

It is the practical use of a classification scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species. Identifying a bacterium usually involves obtaining a pure culture of it and comparing it using various tests to a large number of other species until the corresponding one is found.

In practice, the identification of bacteria requires knowledge of their morphologic, biochemical, physiological, and genetic characteristics. As a science, taxonomy is dynamic and subject to change on the basis of available data.

8. Different taxonomic approaches

Microorganisms are regarded as a collection of evolutionary different organisms. Many different approaches are used in classifying and identifying microorganisms. The characters used to classify bacteria fall into two groups, i.e. traditional (or classical) and genomics (or molecular).

8.1. Classical characters

Classical approaches to taxonomy make use of morphological, physiological, biochemical, ecological, and genetic characteristics. These characteristics have been employed in microbial taxonomy for many years. They are quite useful in routine identification and may provide phylogenetic information as well.

8.1.1. Morphological characteristics

Morphological features are important in microbial taxonomy for many reasons. Morphology is easy to study and analyze, particularly in eukaryotic microorganisms and the more complex prokaryotes. In addition, morphological comparisons are valuable because structural features depend on the expression of many genes, usually genetically stable, and normally (at least in eucaryotes) do not vary greatly with environmental changes. Thus, morphological similarity is a good indication of phylogenetic relatedness. Many different morphological features are employed in the classification and identification of microorganisms: cell shape, cell size, cells arrangement (single or in chains, clusters packets, etc), colonial morphology, ultrastructural characteristics, staining behavior, cilia and flagella, flagella arrangement, mechanism of motility, endospore shape and location, spore morphology and location, cellular inclusions and color.

8.1.2. Physiological and metabolic characteristics

Physiological and metabolic characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes. Many biochemical and physiological characters used to classify bacteria are based on conditions that support growth as: carbon and nitrogen sources, cell wall constituents, energy sources, fermentation products, general nutritional type, luminescence, mechanisms of energy conversion, motility, osmotic tolerance, oxygen relationships, pH optimum and growth range, photosynthetic pigments, salt requirements and tolerance, secondary metabolites formed, sensitivity to metabolic inhibitors and antibiotics and storage inclusions (Fig. 04).

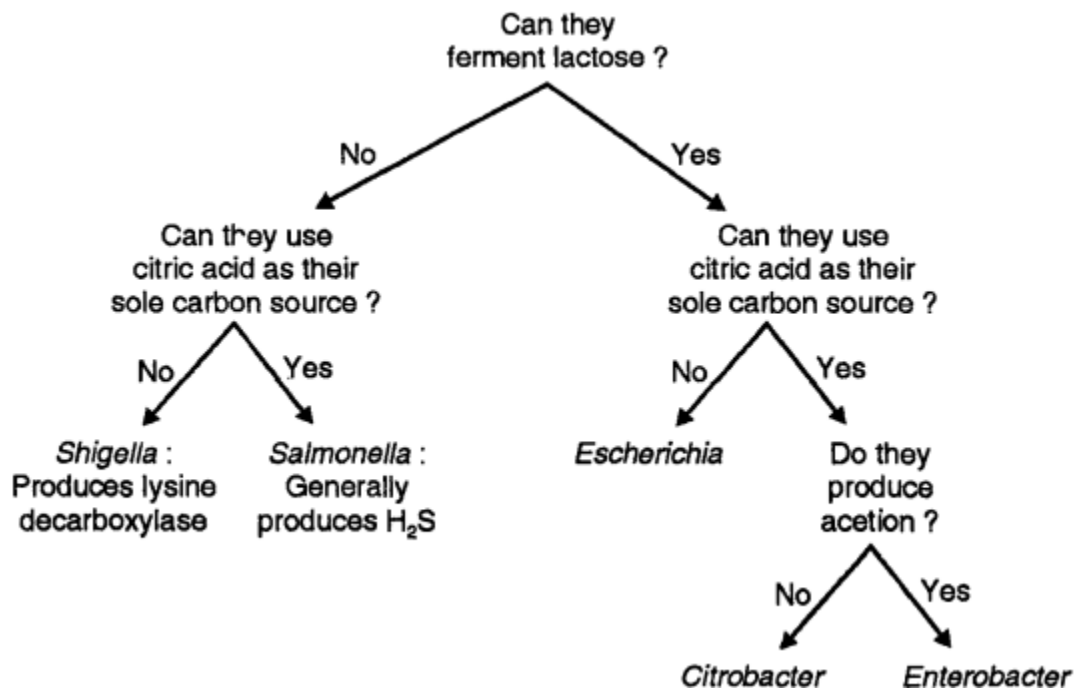


Figure 04: Use of metabolic characteristics to identify selected genera of enteric bacteria.

8.1.3. Ecological characteristics

The ability of a microorganism to colonize a specific environment is of taxonomic value. Some microbes may be very similar in many other respects but inhabit different ecological niches, suggesting they may not be as closely related as first suspected. Some examples of taxonomically important ecological properties are life cycle patterns; the nature of symbiotic relationships; the ability to cause disease in a particular host; and habitat preferences such as requirements for

temperature, pH, oxygen, and osmotic concentration. Many growth requirements are considered physiological characteristics as well (Section 8.1.2).

8.1.4. Serology

The other way to classify and identify bacteria is through serology (the science that studies serum, the noncellular fraction of blood). Serum contains antibodies (protein molecules made in response to infection) and these antibodies are highly specific, they target specific microbes. Thus, antibodies can distinguish between closely related microorganisms and even between strains. Sera that inactivate particular bacteria are called antisera. These antisera are used in a number of ways. For example, they can be used in slide agglutination test to identify particular species or strains. Fluorescent labelled antibodies prepared from antisera are also used in a similar but more sensitive procedure.

8.1.5. Phage typing

Bacteriophages, or phages (viruses that attack bacteria) can also be used to classify bacteria. The pattern of strains attacked by a set of bacteriophages is called phage typing. The diversity of bacterial strains that one bacteriophage will attack (termed the host range) is quite narrow. Only closely related bacterial strains are attacked by the same phages, phage typing is usually restricted to classifying strains within species.

In phage typing, a thin layer of the bacterial strain to be tested is spread on the surface of an agar plate and small drops of suspensions of various phages are placed on the surface. If the bacteria are susceptible to the phages in one or more of the drops they will lyse, producing a clear zone in the lawn (confluent layer of bacterial growth) that forms.

8.1.6. Genetic analysis

Is the study of chromosomal gene exchange through transformation and conjugation because prokaryotes organisms do not reproduce sexually as eucaryotes organisms.

Plasmids are important taxonomically because they can confound the analysis of phenotypic traits. Most microbial genera carry plasmids and some plasmids are passed from one microbe to another with relative ease. When such plasmids encode a phenotypic trait (or traits) that is being used to develop a taxonomic scheme, the investigator may assume that the trait is encoded by chromosomal genes.

8.2. Molecular characteristics

In the 1960s, taxonomists began to develop methods to examine greater portions of the genome. Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids because these are either direct gene products or the genes themselves. Comparisons of proteins and nucleic acids yield considerable information about true relatedness. These molecular approaches have become increasingly, important in prokaryotic taxonomy.

8.2.1. Comparison of proteins

There are several ways to compare proteins. The most direct approach is to determine the amino acid sequence of proteins with the same function. Because protein sequencing is slow and expensive, more indirect methods of comparing proteins frequently have been employed. The electrophoretic mobility of proteins is useful in studying relationships at the species and subspecies level. Antibodies can discriminate between very similar proteins, and immunologic techniques are used to compare proteins from different microorganisms.

Amino acid sequences of proteins are direct reflections of mRNA sequences and therefore closely related to the structures of the genes coding for their synthesis. Comparisons of proteins of different microorganisms are very useful taxonomically.

8.2.2. Nucleic acid base composition

It is, possibly, the simplest technique to be employed in the determination of DNA base composition by calculating (G + C)/(A + T) ratio or **G + C content** (or **CHARGAFF Coefficient**) as follow:

$$\text{Mol\% G + C} = \frac{\text{G + C}}{\text{G + C + A + T}} \times 100$$

- The G + C content can be ascertained after hydrolysis of DNA and analysis of its bases with high-performance liquid chromatography (HPLC), physical methods are easier and more often used.

- The G + C content often is determined from the **melting temperature (T_m)** of DNA.

- The DNA with a greater G + C content have more hydrogen bonds, and its strands separate at higher temperatures, that is, it has a higher melting point.

- DNA melting can be easily followed spectrophotometrically because the absorbance of DNA at 260 nm (UV light) increases during strand separation (Fig. 05). The midpoint of the rising curve gives the melting temperature, a direct measure of the G + C content.

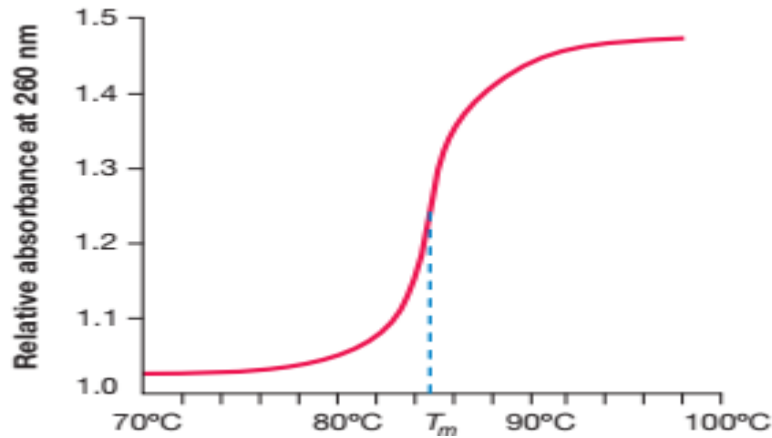


Figure 05: A DNA melting curve. The T_m is indicated

Taxonomically these G + C data are valuable for at least two reasons:

- They can confirm a taxonomic scheme developed using other data. If organisms in the same taxon are too dissimilar in G + C content, the taxon probably should be divided.
- The G + C content appears to be useful in characterizing prokaryotic genera since the variation within a genus is usually less than 10% even though the content may vary greatly between genera (ranging from around 25 to almost 80%).

8.2.3. Nucleic acid hybridization

The similarity between genomes can be compared more directly by use of nucleic acid hybridization studies, this includes DNA-DNA homology (degree of sequence homology). If a mixture of single stranded DNA (formed by heating of ds DNA) is cooled and held at a temperature about 25°C below the T_m , strands with complementary base sequences will reassociate to form stable dsDNA, and the non-complementary strands will remain single (the incubation temperature determines the degree of homologous sequences to form a stable hybrid).

Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in T_m often, but not always, are considered members of the same species. However, DNA preparation from two unrelated bacteria, could not hybridize or they will not form a *stable detectable hybrid*.

8.2.4. Nucleic acid sequencing

Despite the usefulness of G + C content determination and nucleic acid hybridization studies, rRNAs from small ribosomal subunits (5S and 16SrRNA's isolated from 50S and 30S subunits respectively of prokaryotic ribosomes) have become the molecules of choice for inferring microbial phylogenies and making taxonomic assignments at the genus level. Thus, an identical sequence means identical organisms and a similar sequence means closely related organisms.

The rRNA are almost ideal for studies of microbial evolution and relatedness, their functional role is same in all ribosomes. Furthermore, their structure changes very slowly with time. Because the structure of ribosome cannot tolerate much change and still remain functional, rRNA is highly conserved.

Comparative analysis of 16S rRNA sequences from thousands of organisms has demonstrated the presence of **oligonucleotide signature sequences**. These are short, conserved nucleotide sequences that are specific for a phylogenetically defined group of organisms.

8.2.5. Genomic Fingerprinting

A group of techniques called **genomic fingerprinting** can also be used to classify microbes and help determine phylogenetic relationships. Unlike the molecular analyses so far discussed, genomic fingerprinting does not involve nucleotide sequencing. Instead, it employs the capacity of restriction endonucleases (restriction enzymes) to recognize specific nucleotide sequences, and cut DNA molecule at these points. Thus, the pattern of DNA fragments generated by endonuclease cleavage (called restriction fragments) is a direct representation of nucleotide sequence. For example, the enzyme *EcoRI* cuts DNA at the point indicated by the arrow in each sequence (Fig. 06). The comparison of restriction fragments between species and strains is the basis of **restriction fragment length polymorphism (RFLP)** analysis.

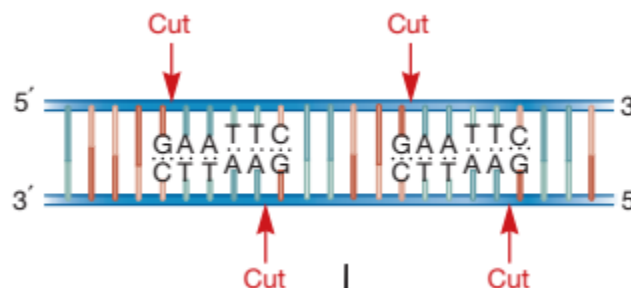


Figure 06: Restriction endonuclease *EcoRI* action.

Because DNA fingerprinting enables identification to the level of species, subspecies, and often strains, it is valuable not only in the study of microbial diversity, but in the identification of human, animal, and plant pathogens as well.

Figure 07 shows the taxonomic utility of several kinds of molecular analyses including protein profiling; with the exception of genome sequencing, it is clear that a combination of approaches is best for identification at the species level or lower.

Family	Genus	Species	Subspecies	Strain
Genome sequencing				
16S rDNA sequencing				
Mol% G+C				
DNA-DNA hybridization				
Multilocus sequence typing				
Whole cell protein profiling				
Genomic fingerprinting				

Figure 07: Relative taxonomic resolution of various molecular techniques.