

Molecular methods of microbial taxonomy

- Microbial taxonomy is a means by which microorganisms can be grouped together.
- Organisms 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.
- There are a number of taxonomic criteria that can be used. For example, numerical taxonomy differentiates microorganisms, typically bacteria, on their phenotypic characteristics.
- Phenotypes are the appearance of the microbes or the manifestation of the genetic character of the microbes.

- Examples of phenotypic characteristics include the Gram stain reaction, shape of the bacterium, size of the bacterium, where or not the bacterium can propel itself along, the capability of the microbes to grow in the presence or absence of oxygen, types of nutrients used, chemistry of the surface of the bacterium, and the reaction of the immune system to the bacterium.
- Numerical taxonomy typically invokes a number of these criteria at once.
- The reason for this is that if only one criterion was invoked at a time there would be a huge number of taxonomic groups, each consisting of only one or a few microorganisms.
- The purpose of grouping would be lost. By invoking several criteria at a time, fewer groups consisting of larger number of microorganisms result.

- The groupings result from the similarities of the members with respect to the various criteria.
- A so-called similarity coefficient can be calculated.
- At some imposed threshold value, microorganisms are placed in the same group.
- A well-known example of taxonomic characterization is the kingdom, division, class, family, genus, species and strain divisions.
- Such a "classical" bacterial organization, which is typified by the Bergey's Manual of Determinative Bacteriology, is based on metabolic, immunological, and structural characteristics.
- Strains, for example, are all descended from the same organism, but differ in an aspect such as the antigenic character of a surface molecule.

- Microbial taxonomy can create much order from the plethora of microorganisms. For example, the American Type Culture Collection maintains the following, which are based on taxonomic characterization (the numbers in brackets indicate the number of individual organisms in the particular category): algae (120), bacteria (14400), fungi (20200), yeast (4300), protozoa (1090), animal viruses (1350), plant viruses (590), and bacterial viruses (400).
- The actual number of microorganisms in each category will continue to change as new microbes are isolated and classified.
- The general structure, however, of this classical, so-called phenetic system will remain the same.

- The separation of the microorganisms is typically represented by what is known as a dendrogram.
- Essentially, a dendrogram appears as a tree oriented on a horizontal axis.
- The dendrogram becomes increasingly specialized—that is, the similarity coefficient increases—as the dendrogram moves from the left to the right.
- The right hand side consists of the branches of the trees.
- Each branch contains a group of microorganisms.
- The dendrogram depiction of relationships can also be used for another type of microbial taxonomy.
- In this second type of taxonomy, the criterion used is the shared evolutionary heritage. This heritage can be determined at the genetic level. This is termed **molecular taxonomy**.

Molecular microbial taxonomy

- Molecular microbial taxonomy relies upon the generation and inheritance of genetic mutations that is the replacement of a nucleotide building block of a gene by another nucleotide.
- Sometimes the mutation confers no advantage to the microorganism and so is not maintained in subsequent generations.
- Sometimes the mutation has an adverse effect, and so is actively suppressed or changed.
- But sometimes the mutation is advantageous for the microorganism.
- Such a mutation will be maintained in succeeding generations.

- Because mutations occur randomly, the divergence of two initially genetically similar microorganisms will occur slowly over evolutionary time (millions of years).
- By sequencing a target region of genetic material, the relatedness or dissimilarity of microorganisms can be determined.
- When enough microorganisms have been sequenced, relationships can be established and a dendrogram constructed.
- For a meaningful genetic categorization, the target of the comparative sequencing must be carefully chosen.
- Molecular microbial taxonomy of bacteria relies on the sequence of **ribonucleic acid (RNA)**, dubbed 16S RNA, that is present in a subunit of prokaryotic **ribosomes**.

- Ribosomes are complexes that are involved in the manufacture of proteins using messenger RNA as the blueprint.
- Given the vital function of the 16S RNA, any mutation tends to have a meaningful, often deleterious, effect on the functioning of the RNA.
- Hence, the evolution (or change) in the 16S RNA has been very slow, making it a good molecule with which to compare microorganisms that are billions of years old.
- Molecular microbial taxonomy has been possible because of the development of the technique of the polymerase chain reaction.
- In this technique a small amount of genetic material can be amplified to detectable quantities

- The use of the chain reaction has produced a so-called bacterial phylogenetic tree.
- The structure of the tree is even now evolving. But the current view has the tree consisting of three main branches.
- One branch consists of the bacteria.
- There are some 11 distinct groups within the bacterial branch.
- Three examples are the green non-sulfur bacteria, Gram-positive bacteria, and cyanobacteria.
- The second branch of the evolutionary tree consists of the Archae, which are thought to have been very ancient bacteria that diverged from both bacteria and eukaryotic organisms billions of years ago.
- Evidence to date places the Archae a bit closer on the tree to bacteria than to the final branch (the Eucarya).
- There are three main groups in the archae: halophiles (salt-loving), methanogens, and the extreme thermophiles (heat loving).

- Finally, the third branch consists of the Eucarya, or the eukaryotic organisms.
- Eucarya includes organisms as diverse as fungi, plants, **slime molds** and animals (including humans).