MICROBIAL CLASSIFICATION AND TAXONOMY



of MICROBIOLOGY

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Taxonomy (Greek taxis, arrangement or order, and nomos, law, or nemein, to distribute or govern) is defined as the science of biological classification. In a broader sense, it consists of three separate but interrelated parts:

- Classification
- > Nomenclature
- ➤ identification

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.

Systematics is often used for taxonomy. However, many taxonomists define systematics in more **general terms as the scientific study of organisms** with the ultimate object of **characterizing and arranging them in an orderly manner**.

Natural classification

- The Swedish botanist Carl von Linné, or Carolus Linnaeus, developed the first natural classification in the middle of the eighteenth century.
- It was based largely on anatomical characteristics and was a great improvement over previously employed artificial systems because knowledge of an organism's position in the scheme provided information about many of its properties.
- For example, classification of humans as mammals denotes that they have hair, self-regulating body temperature, and milk-producing mammary glands in the female.
- Natural classification is applied to higher organisms, evolutionary relationships become apparent simply because the morphology of a given structure.

Traditional taxonomic

Assignment of microbes was **not necessarily rooted in evolutionary relatedness**. Bacterial **pathogens** and microbes of **industrial** importance were historically given names that described the diseases they cause.

Polyphasic taxonomy

In practice, determination of the genus and species of a newly discovered procaryote is based on polyphasic taxonomy.

This approach includes

- > Phenotypic
- Phylogenetic
- Genotypic features.

Phenetic Classification

- This classification system succeeded in bringing order to biological diversity and clarified the function of morphological structures. For example, because motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility.
- Although phenetic studies can reveal possible evolutionary relationships, they do not depend on phylogenetic analysis.
- They compare many traits without assuming that any features are more phylogenetically important than others that is, unweighted traits are employed in estimating general similarity.
- The best phenetic classification is one constructed by comparing as many attributes as possible. Organisms sharing many characteristics make up a single group or taxon.

Phylogenetic Classification

- With the publication in 1859 of Charles Darwin's On the Origin of Species, biologists began developing phylogenetic or phyletic classification systems that sought to compare organisms on the basis of evolutionary relationships.
- The term phylogeny (Greek phylon, tribe or race, and genesis, generation or origin) refers to the evolutionary development of a species.
- Scientists observed differences and similarities between organisms as a result of evolutionary processes.
- The validity of this approach is now widely accepted, and there are currently over 200,000 different 16S and 18S rRNA sequences in the international databases GenBank and the Ribosomal Database Project (RDP-II).

Genotypic Classification

- Currently the genotype of a microbe can be evaluated in taxonomic terms in many ways. In general, genotypic classification seeks to compare the genetic similarity between organisms.
- Individual genes or whole genomes can be compared. Since the 1970s it has been widely accepted that procaryotes whose genomes are at least 70% homologous belong to the same species.
- Unfortunately, this 70% threshold value was established to avoid disrupting existing species assignments; it is not based on theoretical considerations of species identity.

The genetic data obtained using newer molecular approaches often concur with these older assignments.

TAXONOMIC RANKS

- The highest rank is the domain, and all procaryotes belong to either the Bacteria or the Archaea.
- > Within each domain, each microbe is assigned (in descending order) to a
 - Phylum
 - Class
 - Order
 - Family
 - Genus
 - Species
 - Strains

The basic taxonomic group in microbial taxonomy is the species .

Some procaryotes are also given a **subspecies** designation.

> Microbial groups at each level have a **specific suffix indicative** of that rank or level.



Prescott's Principles of MICROBIOLOGY

- A procaryotic species is a collection of strains that share many stable properties and differ significantly from other groups of strains.
- > A strain consists of the descendants of a single, pure microbial culture.
- The definition of a procaryotic species is subjective and can be interpreted in many ways.
- With an increasing amount of genome sequence data, some have argued that the definition of a procaryotic species needs further revision.
- Perhaps a species should be the collection of organisms that share the same sequences in their core housekeeping genes.

Strains within a species may be described in a number of different ways

> Biovars are variant strains characterized by biochemical or physiological differences

Morphovars differ morphologically

> Serovars have distinctive antigenic properties.

- > For each species, one strain is designated as the **Type Strain**
- 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.

A new procaryotic 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.

Questions

Q1. What is the difference between a procaryotic species and a strain?

Q2. Define morphovar, serovar, and type strain.

TECHNIQUES FOR DETERMINING MICROBIAL TAXONOMY AND PHYLOGENY

Many different approaches are used in classifying and identifying microorganisms. For clarity, these have been divided into two groups:

- Classical
- Molecular

Classical Characteristics

Classical approaches to taxonomy make use of

- > Morphological
- > Physiological
- Biochemical
- Ecological
- 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.

Morphological Characteristics

Table 17.2Some Morphological Features Used in Classification and Identification			
Feature	Microbial Groups		
Cell shape	All major groups ^a		
Cell size	All major groups		
Colonial morphology	All major groups		
Ultrastructural characteri	stics All major groups		
Staining behavior	Bacteria, some fungi		
Cilia and flagella	All major groups		
Mechanism of motility	Gliding bacteria, spirochetes, protists		
Endospore shape and loc	ation Endospore-forming bacteria		
Spore morphology and lo	ocation Bacteria, protists, fungi		
Cellular inclusions	All major groups		
Colony color	All major groups		

Prescott's Principles of MICROBIOLOGY

Physiological and Metabolic Characteristics

Table 17.3	Some Physiological and Metabolic Characteristics Used in Classification and Identification			
Carbon and nitrogen sources				
Cell wall constituents				
Energy sources				
Fermentation products				
General nutritional type				
Growth temperature optimum and range				
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				
Storage inclusions				

Ecological Characteristics

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
- Habitat preferences such as requirements for temperature, pH, oxygen, and osmotic concentration

Many growth requirements are considered physiological characteristics as well.

Genetic Analysis

Because most **eucaryotes are able to reproduce sexually**, classical genetic analysis has been quite useful in the classification of these organisms.

Procaryotes **do not reproduce sexually**, the study of **chromosomal gene exchange** through **transformation**, **conjugation**, and **transduction** is sometimes useful in their classification.

Transformation can occur **between different procaryotic species** but only **rarely between genera**. The demonstration of transformation between two strains therefore provides evidence of a close relationship.

Despite the usefulness of transformation, its results are sometimes hard to interpret.

Conjugation studies also yield taxonomically useful data, particularly with the enteric bacteria.

For example, *Escherichia* can undergo conjugation with the genera *Salmonella* and *Shigella* but not with *Proteus* or *Enterobacter*.

These observations fit with other data showing that the first three of these genera are more closely related to one another than to Proteus and Enterobacter.

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.

Thus a microbe's phenetic characteristics are misunderstood and its relative degree of relatedness to another microbe may be overestimated.

For example, hydrogen sulfide production and lactose fermentation are very important in the taxonomy of the enteric bacteria, yet genes for both traits can be borne on plasmids as well as bacterial chromosomes.

Molecular Characteristics

It is hard to overestimate how the study of DNA, RNA, and proteins has advanced our understanding of microbial evolution and taxonomy.

Nucleic Acid Base Composition

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

DNA melting can be easily followed spectrophotometrically because the absorbance of DNA at 260 nm increases during strand separation.



- Procaryotic G+C content is the most variable, ranging from around 25 to almost 80%. Despite such a wide range of variation, the G+C content of strains within a particular species is constant. If two organisms differ in their G+C content by more than about 10%, their genomes have quite different base sequences, indicating that they are not closely related.
- ➢ On the other hand, it is not safe to assume that organisms with very similar G+C contents also have similar DNA base sequences because two very different base sequences can be constructed from the same proportions of A+T and G+C base pairs.

Table 17.4

Representative G + C Content of Microorganisms

Organism	Percent G + C	Organism	Percent G + C	Organism	Percent G + C
Procaryotes		Rickettsia	29–33	Plasmodium berghei	41
Anabaena	39–44	Spirochaeta	51-65	Spirogyra	39
Caulobacter	62-65	Staphylococcus	30–38	Stentor polymorphus	45
Chlamydia	41-44	Streptococcus	33-44	Trichomonas	29–34
Chlorobium	49–58	Streptomyces	69–73	Trypanosoma	45-59
Clostridium	21–54	Sulfolobus	31–37	Volvox carteri	50
Deinococcus	62-70	Thiobacillus	52-68	Eurori	
Escherichia	48-59	Proticto		Agarigus hisporus	44
Halobacterium	66–68	Acetabularia mediterranea	27 52	Aguricus disporus Amanita muscaria	44 57
Methanobacterium	32-50	Aceiabaiana medilerranea	57-55	Amanua muscana Asparaillus piage	57
Micrococcus	64-75	Chlamodomonas	60 68	Asperguius niger	32
Mycobacterium	62-70	Chlaralla	00-08	Canaiaa aibicans	50-50 50-50
Myxococcus	68–71	District	45-19	Coprinus iagopus	32-33
Neisseria	48-56	Dictyostelium	22-25	Mucor rouxii	38
Oscillatoria	40-50	Euglena gracuis	40-33	Neurospora crassa	52-54
Pseudomonas	58-69	Nitzschia angularis	47	Knizopus nigricans	47
Rhodospirillum	62-66	Paramecium spp.	29-39	Saccharomyces cerevisiae	36-42

Nucleic Acid Hybridization

- The similarity between genomes can be compared more directly by use of nucleic acid hybridization studies.
- If the genomes of two microbial isolates are heated to become single-stranded (ss) DNA and then cooled and held at a temperature about 25°C below the Tm , strands with complementary base sequences will reassociate to form stable dsDNA.
- > However, noncomplementary strands will remain unpaired.
- Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in Tm often, but not always, are considered members of the same species.

Table 17.5Comparison of Neisseria Species by DNA Hybridization Experiments				
Membrane-Attached	d DNA ^a	Percent Homology ^b		
Neisseria meningitidis		100		
N. gonorrhoeae		78		
N. sicca		45		
N. flava		35		

Nucleic Acid Sequencing

- In addition, because the ribosome is absolutely necessary for survival and the SSU rRNAs are part of the complex ribosome structure, the genes encoding these rRNAs cannot tolerate large mutations.
- Comparative analysis of SSU rRNA sequences from thousands of organisms has demonstrated the presence of oligonucleotide signature sequences. These are short, conserved nucleotide sequences the signature sequences found in Bacteria are rarely or never found in Archaea and vice versa.
- When comparing rRNA sequences between two microorganisms, their relatedness can be represented by an association coefficient, or Sab value. The higher the Sab values, the more closely the organisms are related to each other. If the sequences of the 16S rRNAs of two organisms are identical, the Sab value is 1.0.
- In fact, rather than using a single gene, five to seven conserved housekeeping genes can be sequenced and compared in a technique called multilocus sequence typing (MLST).

Genomic Fingerprinting



The comparison of restriction fragments of specific genes or DNA fragments is the basis of restriction fragment length polymorphism (RFLP) analysis. Sometimes, rather than determining the nucleotide sequence of the SSU rRNA genes from a group of microbes, the rRNA genes (or other genes) undergo RFLP analysis. That is, the genes are digested by endonucleases and their restriction fragment patterns are compared. When rRNA genes are analyzed in this way, it is called **ribotyping**.

Amino Acid Sequencing

- The sequences of cytochromes and other electron transport proteins, histones and heat-shock proteins, transcription and translation proteins, and a variety of metabolic enzymes have been used in taxonomic and phylogenetic studies.
- A sequence of 20 amino acids has more information per site than a sequence of four nucleotides.
- Protein sequences are less affected by organism-specific differences in G+C content than are DNA and RNA sequences.

Relative Taxonomic Resolution of Various Molecular Techniques

