

Taxonomy and Identification (Classification) of Microorganisms

Taxonomy – Taxonomy may be defined as the science or study of the classification of living organisms. It involves separating living organisms (and fossil forms of preexisting organisms) into groups or categories and developing the criteria to be used for determining which organisms fit into which groups. Grouping or categorizing living organisms allows investigators to study and understand them more readily. The categories used in the classification of organisms are intended to show the **natural relationships** between organisms and to reflect **phylogeny**, i.e., the evolutionary history of organisms.

Recently, new methods for analyzing the biochemical content of organisms have led to the development of new criteria for classification (especially in microbiology), and although this is exciting for taxonomists, it has created inconsistencies in reference sources resulting in considerable confusion for students. It is not unusual to find different authors applying different criteria, and placing the same organisms into different groups. Therefore, if you have studied classification schemas in other classes, it is likely that the one presented in this class will be different. My apologies for the frustration I know this will cause some of you.

The tendency to categorize (vehicles, foods, clothing, etc.) is common to humans and not restricted to biologists; however, much of the terminology associated with taxonomy is new to beginning students and can therefore be intimidating. For this reason, information about specific representative organisms and their taxonomic relationships will be covered in the laboratory (where organisms can be observed first hand). The information presented here is of a more general nature and includes terminology applicable to taxonomy, but also required for understanding other topics introduced later in the semester.

Binomial Nomenclature – Binomial nomenclature refers to the two-part technical name applied to each different type of living organism. It is important to biologists because it provides a system for communicating information about specific organisms named in a language universally recognized and accepted.

The development of the binomial system of nomenclature (binomial nomenclature) is credited to **Carolus Linnaeus** (a botanist/naturalist), in association with his *Systema Natura*, a manuscript containing a classification of living organisms, first published in **1735**. Linnaeus's text contained lengthy descriptions of multiple living organisms, but also included a two-part name for each one, based on key characteristics.

Currently the two-part technical name applied to each different type of living organism includes the **genus name** (which is capitalized) and the **specific epithet** (all lower case). Both names are Latinized and include either Latin or Greek roots providing descriptive information. For example the name *Staphylococcus aureus*, describes a type of organism forming grape-like clusters of spherical-shaped cells, and golden or yellow-colored colonies. Linnaeus's text was in Latin because it was the language used in universities at

the time; however, since Latin is no longer a spoken language, terms and their meanings remain stable and provide the basis for universally accepted scientific communication. Currently, the binomial names of organisms are **italicized when in print and underlined when written by hand**, a convention allowing for easy recognition. The two-part name applied to each type of organism indicates where that organism fits into a larger taxonomic schema as indicated below.

Taxonomic Ranks – Taxonomic ranks are the categories used in the classification of living organisms. These are nested ranks, with each successively lower level being contained within the one above. A group of organisms occupying a specific rank is called a **taxon** (plural = taxa) or **taxonomic group**.

The original taxonomic ranks were as follows:

Kingdoms (singular = Kingdom, the largest or most encompassing)
Phyla (singular = phylum) Sometimes called Divisions
Classes (singular = Class)
Orders (singular = Order)
Families (singular = Family)
Genera (singular = Genus)
Species (the most specific category)

One of several mnemonic forms = Kids playing chase on freeways go splat!

At the time of Linnaeus's work, living organisms were grouped into two broad categories, the **Plantae** (plants) and the **Animalia** (animals). These broad categories were called **Kingdoms**. Since then, a number of classification categories have been added between the levels of kingdom and genus. Similar organisms with the same genus name, or **genera**, are grouped within the same **family**, similar families are grouped within the same **order**, similar orders are grouped within the same **class**, similar classes are grouped within the same **phylum** (or **division**), and similar phyla are grouped within the same kingdom. The criteria or rules used for the classification of living organisms into taxonomic ranks are quite specific and are determined by groups of biologists from around the world. These international groups called congresses meet at varying intervals to determine how plants and animals are to be categorized.

Although most macroscopic organisms can be readily classified into two kingdoms (Plantae and Animalia), microscopic organisms cannot. Following Van Leeuwenhoek's discovery of microscopic life forms, many new organisms were identified that did not meet the criteria for either kingdom. One way to solve this problem was to establish a new kingdom. In 1866, **Ernst Haeckel** proposed a third kingdom be established which he called **Protista**. This kingdom would include all single-celled organisms and those multicellular forms not developing complex tissues. A diverse group of organisms including protozoa, algae, fungi, sponges and slime molds were to be classified within this kingdom, but their relatedness was minimal. In 1957, **Roger Stainer** and his associates used electron microscopy to demonstrate significant differences between prokaryotic and eukaryotic cells, suggesting more than three kingdoms were required. In 1969, **R.H. Whittaker** proposed a five-kingdom system to improve classification. This system included three kingdoms of more complex organisms based on three modes of nutrition. The Animalia ingest food, the Plantae make their own food via photosynthesis, and the Fungi (Myceteae) absorb food in a liquid form. The other two kingdoms, Protista and

Monera, include organisms without complex structures that are separated based on their cell types. Monera are prokaryotic and Protista are eukaryotic. Although the Whittaker five-kingdom system is included in many modern textbooks, it is not without problems. Recent studies based on biochemical analyses indicate considerable variation among eukaryotic microorganisms, and the need for multiple additional kingdoms. In 1978, **Carl Woese** and his associates using biochemical analyses demonstrated significant differences within the kingdom Monera; and prompted the addition of a new taxonomic rank called **Domain** above kingdoms in the taxonomic hierarchy. This work provided evidence that organisms now recognized as Archaea (formerly Archaeobacteria) have multiple important characteristics unlike either bacteria or eukaryotic organisms. The three domains of life currently accepted by most biologists include the **Eukarya** (all organisms with eukaryotic cells), the **Bacteria** and the **Archaea**.

Adding domains to the previously established taxonomic ranks generates a slightly modified hierarchy as shown below:

Domain (plural = Domains, the largest or most encompassing)

Kingdom (plural = Kingdoms)

Phylum (plural = Phyla) Sometimes called Divisions

Class (plural = Classes)

Order (plural = Orders)

Family (plural = Families)

Genus (plural = Genera)

Species (the most specific category)

Mnemonic form = Dumb kids playing chase on freeways go splat!

Since understanding the **phylogeny** (evolutionary history) of life on earth is a major goal of taxonomists, numerous methods have been employed to determine the evolutionary relationships between organisms. Since the 1970s, computer technology and a method called **cladistics** have provided considerable information relative to evolutionary trends. In cladistics, specific features of organisms are used to determine relatedness. A feature that is common to several different types of organisms, but shows variation within them is assigned a value or form called a **character state**. Analysis of the character is then conducted to determine which state is primitive (ancestral) and which is derived (evolved from something else). Finally, the evolutionary relationships determined are portrayed as straight-line diagrams (evolutionary trees) called **cladograms**. Many changes in taxonomy, including the addition of the new taxonomic rank (domain), are due to studies involving cladistics.

Although the primary features used to determine the relatedness between macroscopic organisms were initially based on **morphology** (the study of external features) and mode of reproduction, these are not as useful for the classification of microorganisms. New features such as types of nutrition and metabolism, temperature requirements, gas requirements, pH and salinity preferences, and biochemical properties have proven to be much more useful. Taxonomy is an ongoing science, and despite multiple new discoveries, a complete “picture” of the relatedness between all living organisms has yet to be developed.

Because **viruses are non-cellular entities**, they are not included in any of the taxonomic schemas described above. Viruses are often categorized according to the organisms they

infect, but new taxonomic schemes are being developed to better demonstrate the natural relationships between viruses.

Criteria Useful in the Identification and/or Classification of Microorganisms

Some of the information/terminology included in this section relates to microbial growth and the culture of microorganisms; however, since it also relates to identification and classification, it will be presented here.

1. **Morphology** – Although highly valuable in the classification of multicellular organisms, morphology has limited usefulness when applied to prokaryotes. Many different types of bacteria form colonies and cells with similar morphology even when subjected to various stain techniques. Recall information presented on colony and cell morphology in the laboratory.
2. **Mode of Reproduction** – Variation in reproductive structures/methods is of primary consideration in the classification of plants, animals and fungi; but somewhat less useful in the classification of single-celled organisms. Most single-celled eukaryotes, bacteria and archaea reproduce by means of fission, i.e., one cell divides itself into two daughter cells.
3. **Nutrition and Metabolism** – All living organisms can be categorized on the basis of their nutritional requirements and type of metabolism.

A. Nutritional categories are based on **energy source** and **carbon source**. Organisms can obtain the energy they require either from light or from chemicals. Those using light energy are called **phototrophs** (photo = light) and those using chemical energy are called **chemotrophs** (Chemo = chemical). In this case the root word **troph** refers to activity and organisms can be activated either by light or by chemicals.

Organisms can obtain the carbon they need either from inorganic or organic carbon compounds. Organisms using inorganic compounds as carbon sources are called **autotrophs** (auto = self) while those using pre-formed organic compounds as their source of carbon are called **heterotrophs** (hetero = different). The term troph in this case refers to feeding, so organisms are either feeding themselves or feeding on a variety of different organic materials. By combining energy source and carbon source, we obtain four nutritional categories:

Photoautotrophs = Organisms using light energy and inorganic compounds for carbon.

Photoheterotrophs = Organisms using light energy and organic compounds for carbon.

Chemoautotrophs = Organisms using chemical energy and inorganic compounds for carbon.

Chemoheterotrophs = Organisms using organic compounds for both energy and carbon.

Plants, algae and some bacteria are photoautotrophs, but only prokaryotic cells function as photoheterotrophs or chemoautotrophs. Animals (including humans), fungi, protozoa and many prokaryotes function as chemoheterotrophs, so this category is often subdivided.

Saprotrophs = Chemoheterotrophs using dead or decaying organic materials for nutrients.

These are sometimes called saprophytes or decomposers.

Parasites = Chemoheterotrophs using living organisms as their source of nutrients (some living inside their host and others living outside).

Hypotrophs = Obligate intracellular parasites, i.e., organisms able to grow and reproduce only when inside a living cell. Viruses, some protozoa and some bacteria are hypotrophs.

Carnivores = Chemoheterotrophs obtaining nutrients from meat.

Herbivores = Chemoheterotrophs obtaining nutrients from plant material.

Omnivores = Chemoheterotrophs able to obtain nutrients from both meat and plant material.

B. Metabolism – Metabolism includes all the chemical reactions occurring within living organisms (anabolism and catabolism), and can be categorized as either fermentative or respiratory (oxidative).

Fermentative organisms use organic compounds (usually pyruvic acid) as the final electron acceptors in their metabolic processes.

Respiratory (oxidative) organisms use inorganic compounds (usually molecular oxygen) as the final electron acceptors in their metabolic processes.

- Gas Requirements** – The gas requirements of organisms (based on oxygen utilization) can be useful in their classification as indicated below:
 - Obligately aerobic organisms (obligate aerobes)** = Organisms requiring molecular oxygen for growth and reproduction (metabolic processes).
 - Obligately anaerobic organisms (obligate anaerobes)** = Organisms unable to tolerate exposure to molecular oxygen; oxygen is often toxic to these and they cannot grow in its presence.
 - Facultatively anaerobic/aerobic organisms (facultative anaerobes/aerobes)** = Organisms able to grow and reproduce with or without oxygen available to them.
 - Microaerophiles** = Organisms able to grow best in environments with limited oxygen, as might occur in the mud at the bottom of a pond, lake, sea, etc., or within the gastrointestinal tract.

Although obligately aerobic organisms typically have a respiratory or oxidative metabolism and require oxygen as a final electron acceptor; not all obligately anaerobic organisms are fermentative. Many types of bacteria can use inorganic compounds other than molecular oxygen as final electron acceptors for their respiratory metabolic processes.

- Temperature Requirements** – The temperatures required for optimum growth are variable and can be used to categorize microorganisms as follows:
 - Psychrophiles** = Psychrophiles are cold-loving organisms (psychro = cold, phil = love). These organisms grow best at cold temperatures (between –5 and 20 degrees C).
 - Mesophiles** = Mesophiles are moderate-loving organisms (meso = medium or intermediate) and grow best at moderate temperatures (between 20 and 45 degrees C).
 - Thermophiles** = Thermophiles are warm-loving organisms (thermo = warm) and grow best at warm temperatures (between 45 and 60 degrees C).
 - Hyperthermophiles** = Hyperthermophiles are hot-loving organisms and grow best at hot temperatures, e.g., above 60 degrees C. Hyperthermophiles living in hot springs grow at temperatures above 90 degrees C.Organisms can also be described relative to their temperature tolerance, i.e., ability to survive or tolerate exposure to temperature extremes. Organisms that can tolerate exposure to extreme cold are said to be **psychroduric**. They cannot grow at these temperatures, but do not die either. Most bacteria are psychroduric and can be maintained in a viable state at –70 degrees C. Organisms that can tolerate exposure to heat are said to be **thermoduric**. They cannot necessarily grow in hot environments, but are not killed by exposure to them. Endospores are thermoduric.
- Acidity Vs Alkalinity or pH Requirements** – Although most organisms grow best in neutral environments (pH between 6.5 and 7.5), some prefer acidic environments, and others prefer alkaline. Many types of culture media contain **buffers** (substances that resist pH change) to

help stabilize the pH or **pH indicators** (substances that change color in response to changes in pH) to indicate the presence of acidic or alkaline metabolic end products. Organisms that grow best in acidic environments are called **acidophiles**, but are relatively rare. Highly acidic or alkaline environments tend to inhibit microbial growth because cellular enzymes fail to function under these conditions.

7. **Osmotic Pressure Requirements** – The effective osmotic pressure (**tonicity**) of an environment is influenced by the solute concentration present, and can significantly impact microbial growth.

Isotonic environments (iso = same) contain solute levels similar to protoplasm, so cells placed into them will experience neither a net gain nor net loss of water.

Hypotonic environments (hypo = under, beneath, less than or too little) contain lower levels of solute than protoplasm and will cause cells placed into them to gain water. Microorganisms equipped with **cell walls** (e.g., algae, fungi, bacteria and archaea) or **contractile vacuoles** (many types of fresh water protozoa) can live comfortably in hypotonic environments. Organisms lacking these protective structures will tend to take on water (via osmosis) until they explode.

Hypertonic environments (hyper = over, above, too much or excessive) contain higher levels of solute than protoplasm and will cause cells placed into them to lose water. Hypertonic environments containing high levels of salt or sugar are often used to preserve foods, i.e., inhibit microbial growth within those foods.

Organisms capable of growing and reproducing in environments containing high levels of salt are called **halophiles**. These may be categorized as **extreme halophiles/obligate halophiles** (those requiring high levels of salt for growth) or **facultative halophiles** (those capable of growing with or without salt).

8. **Environmental Relationships** – The types of environmental relationships microorganisms form with other organisms can be useful as criteria for classification; however, these relationships are often not thoroughly documented nor understood.

Symbiosis – Symbiosis is a condition or circumstance existing when two or more different types of organisms are living together in a close association. Although once thought to be unusual, symbiosis is now recognized as a common occurrence, essential to ecosystem function.

Pathogen Vs Host – Pathogens growing within a host benefit from host resources, but the host is harmed, and sometimes killed. Microorganisms capable of causing infection and disease in humans, domestic animals and plants used in agriculture have been extensively studied, but represent an extremely small percentage of the total.

Parasite Vs Host – Parasites also benefit from their hosts without giving in return. Organisms capable of parasitizing humans and other animals have been studied extensively because some cause disease and others serve as vectors involved in the transmission of disease-causing agents.

Mutualistic relationships (mutualism), i.e., those involving organisms in mutually beneficial arrangements are the most common form of symbiotic relationships. Even pathogens and parasites can be considered beneficial in the sense that they help prevent population overgrowth and maintain balance within ecosystems, a concept foreign/repugnant to most humans.

9. **Biochemical Analysis** – Biochemical analysis allows for a more technical evaluation of the relationships existing between organisms and has become the method of choice for the classification of bacteria and archaea. Various subcategories exist as follows:
- A. **Enzymatic Testing** – The types of enzymes organisms produce can be determined by testing their ability to catabolize various materials and/or to form specific end products. Enzymatic testing will be used extensively during the identification of Physiological Unknown No. 1.
 - B. **Chromatography** – Various applications of chromatography can be used to identify specific chemical constituents of cells, e.g., cell wall lipid or amino acid content, membrane protein content, or the presence of specific pigments.
 - C. **Serology** – Serology is the science or study of antibody and antigen interactions in vitro, and has multiple applications in the detection, identification and classification of microorganisms. Microorganisms are **antigenic**, i.e., are perceived by the body as foreign agents (**antigens**), and typically stimulate the production of immune proteins called **antibodies**. Because the interactions between antigens and antibodies are quite specific, and because antibodies can bind with antigens, it is possible to use known types of antibodies to detect or identify specific types of antigens. Several different types of serological reactions will be explained and demonstrated in the laboratory.
 - D. **Phage Typing** – Phage typing (bacteriophage typing) involves the use of viruses called bacteriophages. Like antibodies, these will recognize and bind with specific types of bacteria; however, unlike antibodies, they cause infection typically resulting in cell death. Because these viruses are host-specific, known types of virus particles can be used to identify unknown types of bacteria. Phage typing will be explained and demonstrated in the laboratory.
 - E. **Nucleic Acid Analysis** – The analysis of nucleic acids, DNA and RNA, can provide considerable information useful in the identification and classification of microorganisms. Techniques commonly used in nucleic acid analysis include:
 - 1. **Percent base composition (G + C or A + T)** – Organisms with identical percentages in base composition may or may not be closely related, but organisms with very different percentages in base composition are not related.
 - 2. **Nucleic Acid Hybridization** – Hybridization, the ability of two nucleic acid strands to form hydrogen bonds with one another, has multiple applications including PCR and DNA chip technology.
 - 3. **Polymerase Chain Reaction (PCR)** – The polymerase chain reaction involves hybridization and can be used to amplify DNA or RNA in vitro.
 - 4. **Gel Electrophoresis** – Gel electrophoresis can be used to separate DNA or RNA fragments on the basis of size by exposing them to an electric field.
 - 5. **DNA Fingerprinting or RFLP analysis** – Fragments of DNA generated by restriction endonuclease digestion will form patterns when subjected to electrophoresis. These patterns are called DNA fingerprints or RFLP patterns.
 - 6. **Nucleotide sequencing** – Determining the sequence of nucleotides in a strand of DNA or RNA can yield information highly significant to identification and classification.

Note – Methods 2, 3, 4, 5 and 6 will be covered more extensively in the laboratory where they will be used in the identification of Physiological Unknown No. 2.

- F. **Protein analysis** – The analysis of proteins other than antibodies can also be useful in the identification and classification of microorganisms. Some methods involved include:
1. **Gel electrophoresis** – Similar to methods used with nucleic acids.
 2. **Amino acid sequencing** – Determining the sequence of amino acids present in a protein can be useful in determining protein function and sometimes protein origin. For example, the origin of prions (infectious protein particles) was determined using amino acid sequencing in conjunction with nucleic acid analysis.

According to the taxonomic system currently used by biologists, living organisms are grouped relative to similar characteristics, i.e., they are categorized according to specific criteria as described above. Under the binomial system of nomenclature (**binomial nomenclature**), each different type of organism is identified with a two-part technical name (scientific name) indicating its **genus** and **species**. In the case of multicellular, eukaryotic organisms, a species is defined as a group of closely related organisms that will breed among themselves. The classification of such organisms is therefore based largely on morphology and mode of reproduction. In the case of prokaryotes a species can be defined simply as a population of cells with similar characteristics (a bacterial culture containing only one population of organisms is considered a pure culture and contains only one species). Because most prokaryotes have similar morphology and mode of reproduction, prokaryotic taxonomy is based primarily on other criteria, with biochemical analysis being most important.

Once the criteria for classification have been determined, it is necessary to devise methods for comparing the characteristics of specific groups with those of newly discovered organisms. Two methods commonly used for making such comparisons involve the use of **dichotomous keys** and **cladograms**. Dichotomous keys allow investigators to identify organisms by answering a series of questions, each with two possible answers (dichotomous = cut in two). After answering one question, the investigator is directed to answer a second, a third, and so on until the identification is made. Although useful for identification, dichotomous keys provide little information about the evolutionary relationships between organisms. **Cladograms** (clado = branch) are branching tree-like patterns developed through cladistic analysis and typically indicate degrees of relatedness between organisms based on specific criteria. **Cladistics** is a method for hypothesizing the evolutionary relationships among organisms and classifying organisms based on these relationships. Most cladograms currently being made for bacteria are based on r-RNA gene sequence analysis and provide significant information about prokaryotic **phylogeny** (evolutionary history). Complex cladograms hypothesizing the evolutionary relationships between multiple different types of currently existing organisms and their ancestral forms are called **phylogenetic trees**.

Armed with new methods of biochemical analysis and computer technology, modern biologists are attempting to use the information gained through cladistic analysis to reconstruct the pattern of events leading to the distribution of life on our planet. They are attempting to understand the evolutionary relationships between all living organisms and to determine the mechanisms of evolution involved in their origins. This branch of science is called **phylogenetic systematics**, and has been applied extensively to the classification of prokaryotic organisms, i.e., archaea and bacteria.