INTRODUCTORY BIOLOGY AND MICROBIOLOGY

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Introduction

- Definition
  - Microbiology is defined simply as the study of microorganisms, with "micro" meaning small, and "biology," referring to the study of living things.
  - The microorganisms studied vary widely and the field of microbiology is broken down into many subfields of study.

- The field of microbiology is critical to human beings, not only due to the infectious diseases caused by these microbes, but because "good" microorganisms are necessary for us to live.
- Microorganisms, or "microbes" are small living things. Most of these organisms cannot be seen by the naked eye, and until the invention of the microscope.

- Microbes are found nearly anywhere on earth. They are found in boiling pools of water in yellowstone and in volcanic vents at the lowest depths of the sea.
- They can live in salty water and some need oxygen to grow while others do not.

Classification of Microorganisms in Microbiology

- There are many different ways in which scientists have classified, and in doing so tried to make sense, of the millions of microbes in the world.
- One of the ways microbes are classified is by whether or not they have cells, and if so, how many. Microorganisms may be:

1. Multicellular vs unicellular vs acelluar

- **Multicellular** - Having more than one cell.
- **Unicellular** - Having a single cell.
- **Acellular** - Lacking cells, such as viruses and prions. (There has been debate over whether viruses are really living things, as they cannot survive outside of a host, and prions are usually referred to as "infectious proteins" rather than microbes.)

Eukaryotes vs prokaryotes

- Another way in which microorganisms are classified has to do with the type of cell. These include eukaryotes and prokaryotes:
- Eukaryotes are microbes with "complex cells" which have a true nucleus and membrane bound organelles. Examples of eukaryotes include helminths (worms,) protozoa, algae, fungi, and yeasts.
- Prokaryotes are microbes with "simple cells" which do not have a true nucleus and lack membrane bound organelles. Examples include bacteria.

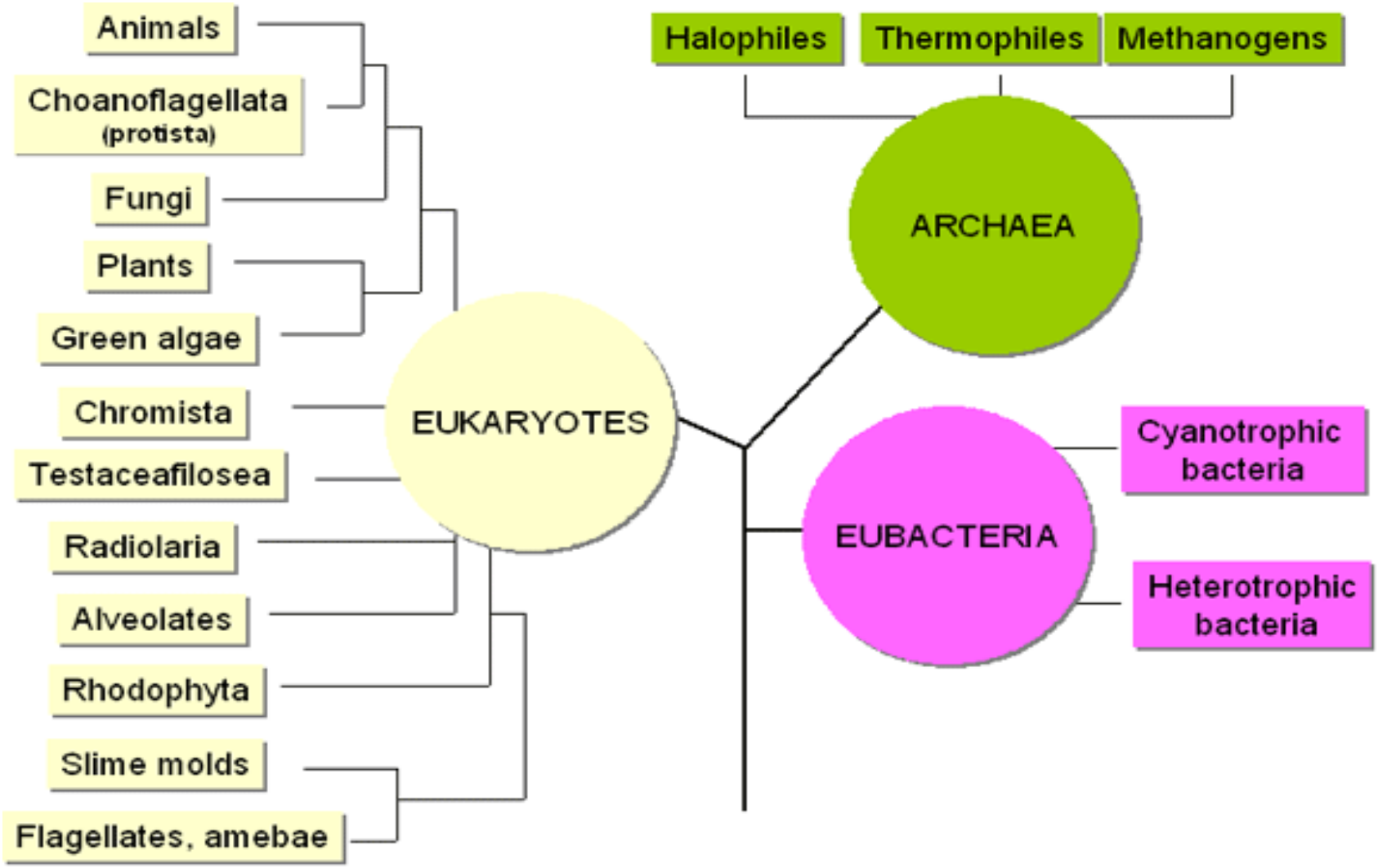
The major classes of microorganisms

- Microorganisms can be classified into the following large biological groups:
  - Protozoa;
  - Algae;
  - Fungi,
  - Slime moulds
  - Bacteria,
  - Archae and
  - Viruses.
  - Prions

- Algae (excluding blue green algae), protozoa, slime moulds and fungi have similar type of structure and organization and are called Eukaryotes.
- Bacteria (including mycoplasma, rickettsia and chlamydia) blue – green algae also have similar form of cellular organization and are called Prokaryotes.

- In the phylogenetic tree there is also another group distantly related to prokaryotes. This is the Archaea (have rRNA similar to those found in Bacteria and Eukaryotes).
- It is therefore believed that Prokaryotes, Archaea and Eukaryotes shared a common ancestor with an conserved DNA that was passed on 3 billion years ago.

The Three-Domain Evolution System



History of Microbiology

- Obviously human have had to deal with microbes even before the recorded history. The first record of human using comes from ancient tablets from mid east.
- Babylonians were using yeast to make beer over 8000 years ago and acetic acid bacteria to make vinegar over 6000 years ago.
- About 5000 years ago, Persia (Now Iran) region recorded the wine making.

- The Romans had God for that were specific for microorganisms. The roman God of mold and mildew was “Robigus” and “Robigo” which means crop rust.
- Rust is one of the plant disease caused by fungus. God Robigus was very much feared because of crop lost.
- About 2000 years ago, Romans proposed that diseases were caused by tiny animals. But, fundamentalist religions had a strong hold over the progress.
- The real microbiology history starts from 1600s, when people began to make crude lenses and microscopes.

Phases of History of Microbiology

- History of microbiology can be discussed in three phases:
  - Experience phase
  - Experimental phase
  - Modern phase

Highlights in the Experience phase of Microbiology

- Bubonic Plague epidemic of mid 1300's, the "Great Plague", reduced population of western Europe by 25%.
- Plague bacterium was carried by fleas, spread from China via trade routes and poor hygiene. As fleas became established in rat populations in Western Europe, disease became major crisis.

- Smallpox and other infectious diseases introduced by European explorers to the Americas in 1500's were responsible for decimating Native American populations.
- Example: In the century after Hernan Cortez's arrival in Mexico, the Aztec population declined from about 20 million to about 1.6 million, mainly because of disease.
- In 1625 and 1630 Francesco Stelluti made the first microscopic (Galileo’s microscope) observation on bees and weevils.

- In 1546 Girolamo Fracastoro suggested that diseases were caused by invissible creatures.
- In 1665 – Robert Hook drew a microorganism like on a Micrographia.

Highlights in the Experimental phase of Microbiology

- To see microbes, you need a microscope. The first microscope was invented by Antony van Leeuwenhoek (1632-1723), a Dutch businessman.
- Leeuwenhoek took up lens grinding to make magnifiying glasses so he could examine fine weave of fabrics. In testing his lenses, he discovered many small creatures he called "animalcules" in samples such as pond water. His best lenses could magnify 300-500X

- Leeuwenhoek microscopes were crude, relied on a single lens held in a metal plate.
- Leeuwenhoek described many previously unseen life forms, including different forms of bacteria, mold spores, etc.
- Leeuwenhoek reported discoveries to Royal Society from 1670's on, firmly established existence of microbes.

Theory of spontaneous generation

- Many people believed that microbes arose from simple non-living matter by the process of spontaneous generation.
- This notion had been posited by Aristotle (382-322 B.C.) and other Greek philosophers to explain decay and appearance of animals such as flies and frogs, and was widely held as common sense even in 1700's and 1800's.

- In 1688 Francisco Redi (1626-1697) refutes SG of maggots. He demonstrated that flies did not arise spontaneously from rotting meat by simple experiment.
- If jar of meat was covered by fine muslin, maggots did not arise. However, the simpler life forms discovered by Leeuwenhoek lacked visible complexity, and most people still believed these could arise spontaneously.

- John Needham (1731-1781), a Scottish clergyman and naturalist claimed existence of a "life force" present in inorganic matter that could cause spontaneous generation.
- One of his more convincing demonstrations was to boil some soup (briefly), pour into clean flasks with cork lids, and show that microbes would soon arise.
- Lazzaro Spallanzani (1729-1799) claimed Needham's organisms came from heat-resistant microbes. If flasks were boiled long enough (1-2 h), nothing grew. But Needham countered that prolonged heating destroyed the "life force".

- In 1796 Edward Jenner develops a vaccine against smallpox.
- In 1859 Louis Pasteur disapproves the theory of spontaneous generation of microorganisms. Pasteur filtered air through cotton to trap airborne materials, then dissolved the cotton and examined the particulate matter under a microscope; many bacteria and spores of other life forms such as molds were present.

- Since most skeptics kept arguing that overheating killed the life force present in air, Pasteur developed and ingenious experiment using a swan neck flask that allowed fresh air to remain in contact with boiled materials.
- The long passageway prevented airborne microbes from reaching the nutrient liquid, without impeding access to air. This experiment brought into existence the germs theory of disease (means germs are responsible for the disease not the inert mater) and ended the SG theory.

Other contributions of Louis Pasteur

- Included:
  - Discovered fermentation of fruit to alcohol by microbes.
  - Sorted different microbes giving different taste of wine.
  - He selected a particular strain (Yeast) for high quality wine.
  - He developed a method to remove the undesired microbes from juice without affecting its quality. Heating the juice at 62.8℃ for half-an hour did the job. This technique is called as pasteurization.

- He discovered that parasites (protozoa) causing pebrine disease of silk worm. He suggested that disease free caterpillars can eliminate the disease.
- He isolated the anthrax causing bacilli from the bloods of cattle, sheep and human being.
- He also demonstrated the virulence (ability of microbe to cause disease) of bacteria.
- He developed vaccine (a killed or attenuated microbe to induce the immunity) against rabbis from the brains and spinal cord of rabbit.

- **John Tyndall (1820 -1893)**
  - Proved that dust carries the germs and if no dust in the air, the sterile broth remained free of microbial growth for indefinite period.
  - He also developed a sterilization method “Tyndallization”, referred as intermittent or fractional sterilization. The subsequent cooling and heating by steam for 3 days removes germs and their spores.

- **Martinus Willium Beijerinck (1851 – 1931).**
  - Developed the enrichment technique to isolate various group of bacteria.
  - He isolated sulphur reducing bacteria and sulphur oxidizing bacteria from soil.
  - He also isolated free-living nitrogen fixing bacterium, Azotobacter from soil,
  - He identified root nodulating bacterium e.g. Rhizobium, Lactobacillus, green algae.
  - He confirmed that Tobacco mosaic virus causes disease and it incorporated in the host plant to reproduce.

- **Sergei Winogradsky (1856 – 1953).**
- The following are the contributions of Winogradsky to soil microbiology.
  - Microorganisms involved in N, C, and S cycle.
  - Documented nitrification process in soil.
  - Described autotrophic nutrition of bacteria.
  - Chemolithotrophic nutrition of soil bacteria.
  - Discovered anaerobic nitrogen fixing bacterium *Clostridium pasteurianum*.

- Robert Koch
  - In 1876 Robert Koch demonstrates anthrax is caused by Bacillus anthracis.
  - In 1880 Laveran discovers Plasmodium, the cause of malaria
  - In 1881- Koch cultures bacteria on gelatin and Pasteur develops anthrax vaccine (Ref: Prescott et al.,2008. 7th Ed. Pg 4 - 6)

Koch's Postulates

- The microbe must be present in every case of the disease but absent from healthy organisms.
- The suspected microorganisms must be isolated and grown in pure culture.
- The same disease must result when the isolated microorganism is inoculated into a healthy host.
- The same micro-organisms must be isolated again from the diseased host.

Koch's Experimentation

- Koch developed a staining technique to examine human tissues. *M. Tuberculosis* cells could be identified in diseased tissues.
- Koch grew *M. tuberculosis* in pure culture on coagulated blood serum
- Koch injected cells from the pure culture of *M. tuberculosis* in guinea pigs. The guinea pigs subsequently died of tuberculosis.
- Koch isolated *M. tuberculosis* from the dead guinea pigs and was able to again culture the microbe in pure culture on coagulated blood serum.

Koch's Molecular Postulate

- The virulence trait under study should be associated much more with pathogenic strains of the species than non pathogenic strains
- Inactivation of the gene or genes (by mutation or knockout) associated with the suspected virulence trait should substantially decrease pathogenicity.
- Replacement of the mutated gene (or knockout) with the normal wild-type gene should fully restore pathogenicity.
- The gene should be expressed at some point during the infection or disease process.
- Antibodies or immune system cells directed against the identified gene products should protect the host.

- **Joseph Lister (1878)**
  - Developed pure culture technique. Pure culture refers to the growth of mass of cells of same species in a vessel.
  - He developed the pure cultures of bacteria using serial dilution technique.
  - He also discovered that carbolic acid could be used to disinfect surgical equipments and dressings. This led to the reduction of post-operational deaths/infections.

- **Walther Hesse and Fannie E. Hesse (1883).**
  - They used agar instead of gelatin for preparation of media. Agar goes to solution at 100°C and solidifies at 45°C. Till now this has not been replaced by any other substance.

- **Alexander Fleming (1928)**
  - Identified *Penicillium notatum* inhibiting *Staphylococcus aureus* and identified the antibiotic as pPenicillin.
  - In 1929 he discovered that antibiotic penicillin was an important milestone in medical microbiology.
  - He found that some natural substances such as Saliva,Nasal mucous had antimicrobial activity.
  - He worked on Staphylococcus aureus and reported that penicillin inhibited the growth of *Staphylococcus aureus*.
  - Florey and Chain purified penicillin.

- **Selman A Waksman**
  - 1927- Wrote the book on Principles of soil Microbiology.
  - In 1939 Waksman and his colleagues undertook a systematic effort to identify soil organisms producing soluble substances that might be useful in the control of infectious diseases, what are now known as antibiotics.
  - Between 1940 – 1949 he identified Streptomycin antibiotic from soil bacterium. The group included actinomycin in 1940, streptomycin in 1944, and neomycin in 1949.

Some Highlights of Modern phase

- In 1982 – Recombinant DNA technology and Hepatitis B vaccine developed.
- In 1983 – HIV isolated and identified and PCR techniques developed.
- In 1986 – First vaccine developed by use of genetic engineering approved for human use.
- In 2003 – SARS outbreak in China.
- In 2005 Super resistant HIV strain isolated from New York City.

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