

Introduction to Microbiology

Microbiology:

- Microbiology is the discipline of science which deals with the study of organisms that require magnification to be observed (>.1mm)
- Microbiology extends to all phases of biology and is the central point of the contemporary fields of biotechnology and genetic engineering.
- Microbiology employs techniques such as (1) sterilization (2) the use of culture media – that necessary for isolation and growth of microorganism.

What are microorganisms?

- They are generally smaller than human eye can detect and belong to each of the five kingdoms:
Monera, Protista, Fungi, Plantae, and Animalia.
Microbiologist primarily study the first three kingdoms
- The subjects of microbiology bacteria, algae, fungi, and protozoa which are cells and viruses which are not cells.
- The majority of microbes exist as single cells or clusters of single cells; however some are multi-cellular existing as filamentous multicells. These organisms are not as complex as animals and plants.

Two Basic Types of Microorganisms:

- *Prokaryotic and Eukaryotic*
- Pro (before) caryos (nucleus)
- Eu (true) caryos (nucleus)

Prokaryotes include the bacteria and cyanobacteria which were formerly classified as blue-green algae.

- They possess a simple architecture that does not contain sub-cellular organelles.
- The typical size of a prokaryote is about 1µm diameter.

Eukaryotic organism possess a complex cellular structure.

- Contain membrane-bound organelles such as mitochondria, lysosomes, endoplasmic reticulum and golgi bodies.

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Protista

Protists are like single-celled animals. The most common organisms within this group are the algae.

- Algae are a diverse group of microorganism that range in size from microscopic single cells to very large, macroscopic seaweeds and fresh water filaments.
- Algae are plant-like in that they contain chlorophyll and are capable of converting light to energy through photosynthesis.

Fungi

- Fungi are eukaryotic microorganisms.
- They are not photosynthetic and include yeasts, molds, and mushrooms.

Viruses

- Represent a unique class of microbes
- They are considered neither prokaryotic nor eukaryotic.
- ***They are not even considered CELLS.***
- They are informational parasites, a piece of bad news wrapped up in a protein.
- They are only considered to be “alive” when they are inside another living cell
- They are described in terms of their *transmissible state as a virus particle.*
- Viruses exist for every group of organism known (including bacteria) and they are typically less than 0.2 um in diameter.

HISTORY OF MICROBIOLOGY

The existence of microorganisms and their relationship to disease was suspected, long before they could actually be seen, but real **physical evidence** was not available until the invention of the microscope.

In 1546 **GIROLAMO FRACASTORO (1478-1553)** published **De Contagione Et Contagiosis Morbis Et Eorum Curatione**

“ On contagion, contagious disease and their cure”

In this document he suggested that Disease was caused by invisible creatures, which could be placed into three categories

- I Those that infect by contact
- II Those that infect by contact and Fomites
He defined fomites as things such as clothes , linens etc. which were not themselves corrupt but could foster the essential seeds of contagion and thus cause contagion.
- III Those that act not only by contact , fomites, but could also be transmitted to a distance

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[Fracastoro's descriptions are remarkably accurate]

Development of microscopy began with early studies of the properties of light and how it can be manipulated with lenses.

circa 1600, Hans and Zaccharias Janssen (father and son): invention of the compound microscope using crude convex lenses

Willebrord Snell in 1621 discovered the law of refraction, resulting in improvements in lens grinding techniques.

In 1665 Robert Hooke made the first microscopic examination of cells in tissue, and published the first description of the fruiting structures of molds.

In 1666, Isaac Newton discovered the light spectrum.

In 1673 Antony van Leeuwenhoek discovered microorganisms, which he called "animalcules"

- Leeuwenhoek's microscopes were able to achieve 50-300X magnification, which enabled him to visualize a variety of bacteria and protozoa
- He communicated his findings to the Royal Society, but kept his techniques for grinding lenses and making observations a secret. Consequently his contemporaries had a very difficult time repeating his results.

The Issue of Spontaneous generation

Spontaneous generation refers to the ancient belief ***that living organisms could arise spontaneously from nonliving matter***. Ironically, this philosophy was based on direct observation rather than superstition .

flies and maggots from decaying meat
eels and frogs from mud
mice from rotting grain.

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The Greek philosopher Aristotle believed insects and other small animals had to arise from spontaneous generation because he was unable to observe organs (including reproductive organs)

This belief prevailed until 1665, when ***Francesco Redi***, an ***Italian physician***, attempted to address the question of Spontaneous Generation experimentally.

He made careful observations of fly eggs and maggots in decaying meat. Concluded spontaneous generation was not occurring.

Decaying Meat Experiment

Placed meat in three different containers:

- 1) Uncovered Meat
 - 2) Meat covered with paper
 - 3) Meat covered with a fine gauze that excluded flies
- Flies laid eggs on the uncovered meat and maggots developed
 - The other two pieces of meat did not produce maggots spontaneously.
 - But flies were attracted to gauze-covered container and laid their eggs on the gauze.
 - These eggs produced maggots
 - Thus the generation of maggots by decaying meat resulted from the presence of fly eggs.
 - ***MEAT*** did not spontaneously generate maggots as previously believed.

THEREFORE DISPROVING THE SPONTANEOUS GENERATION!

As a result of Redi's experiments, ***spontaneous generation was discredited for larger forms of life in favor of the theory of biogenesis (Life from Life)***

Nevertheless, spontaneous generation was still believed to hold true for the ***newly discovered microorganisms***, primarily because they were considered to be too simple to reproduce themselves

Moreover, There were numerous technical difficulties associated with setting up experiments to disprove spontaneous generation.

Scientists at this time often cited the following observations as proof of spontaneous generation...

- Boiled extracts of hay or meat (infusions) would eventually give rise to microbial growth after sitting for a while.

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John Needham and Comte de Buffon (1749) conducted experiments that defended spontaneous generation

- They boiled mutton broth and tightly capped the flasks, eventually the broth became cloudy with “infusoria”
- They believed that organic matter, in this case the mutton, contained a life force that could confer life on non living matter

Lazzaro Spallanzani (1776) challenged Needhams results. He showed that prolonged heating prevented the appearance of microorganisms, and that the amount of heating required to prevent growth was highly variable.

- Spallanzani also used sealed flasks to exclude the air, which he thought was carrying contaminating microorganisms into the infusion.
- There were considerable problems with these Spallanzani’s experiments
- Other researchers were not as meticulous, thus his results were not as reproducible.
- His critics argued that a component of the air was required for spontaneous generation to occur, and he essentially removed air from the system.
- His critics also claimed that prolonged heating destroyed a component in the infusion that was required for spontaneous generation to occur.
- Each of these are valid arguments that needed to be addressed.

In 1861, Louis Pasteur devised an experiment that would settle the controversy.

Pasteur first filtered air through cotton and found a particulate matter that resembled plant spores

If the cotton was placed into a “sterile” medium, growth occurred

He then demonstrated that ***swan-necked flasks*** containing a nutrient broth that had previously been heated would remain sterile, even with open necks.

- He placed nutrients solutions in flasks
- Heated their necks in a flame
- Drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere.
- Boiled the Solution for a few minutes and allowed them to cool.
- ***No growth took place***

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Pasteur concluded that the contaminating particles in the air were trapped on the walls (necks) of the curved irregular flasks.

- If the necks were broken, growth occurred.
- In 1877, **John Tyndall**, demonstrated that dust did carry germs and that if dust was absent, broth remained sterile even if directly exposed to air. Providing the final blow to spontaneous generation.

Pasteur believed that discoveries of science should have practical applications.

In 1866 he responded to a friend's request to help determine why some of the local wines were turning sour.

- Pasteur used his microscope to observe both normal and sour wines.
- In each case he consistently observed large numbers of oval microscopic bodies (yeasts).
- He mixed grape juice + Yeast in a flask
- HEAT
- Plugged flask
- **No fermentation occurred**
- Added back the yeast
- **Fermentation occurred**

Fermentation is defined as: decomposition of plant and animal tissues (carbohydrates) into alcohols and organic acids.

- He concluded that the yeast cells were necessary for the fermentation product
- He also noted that the sour wines differed from the normal wines in that they were heavily laden with microscopic rod shaped organisms (bacilli)
- He hypothesized that if the bacteria were removed, the wine would not sour

Grape + Bact+ Yeast

HEAT

NO FERM

ADD YEAST

WINE

- These experiments indicated that bacteria were the agents of chemical change

I should also point out that in 1887, Eduard Buchner demonstrated that soluble extracts of the yeast cells were able to produce the same fermentation reactions. This was the first experimental proof that **enzymes** were the catalysts of the fermentation reactions

Pasteur's experiments forced physicians to reevaluate their opinions regarding the role of microorganisms in disease.

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Many physicians had long noted the presence of bacteria in the blood and wounds (Pus) of dead patients and animals.

The standard dogma was that the bacteria were an effect of the disease (produced by the disease) NOT the cause of it.

Pasteur showed that bacteria were the cause of chemical reactions, might they also be the cause of disease?

The Germ Theory of disease

Ancients believed that disease was the result of a divine punishment

Hippocrates (ca. 390 BC) believed that disease was a natural phenomenon, caused by inhaling bad air that was polluted with bad vapors or (miasms)

The Italian physician Girolamo Fracastoro proposed his theory of contagions.

Several scientists and philosophers had begun to speculate that these vapors, fomites, and contagions might in fact be microorganisms, however there was never any experimental evidence available to support these theories.

In 1836 Agostino Bassi was the ***first to show that microbes were the cause of disease*** when he demonstrated that a ***silkworm disease was caused by a fungus***.

In 1845, M. J. Berkely demonstrated that the ***great Potato Blight in Ireland*** was caused by ***a fungus (Phytophthora infestans)***.

In spite of these observations, most physicians remained skeptical that human diseases were caused by microorganisms.

In 1943 Oliver Wendall Holmes published a paper on Puerpeal (PURE-PER-AL) sepsis which afflicted mothers during childbirth. Holmes reported that it was much safer to deliver a baby at home than in a hospital... where physician-handling contribute to the disease.

1845, Ignaz Semmelweiss was ridiculed for insisting that physicians wash their hands before working with pregnant women.

- The residents frequently handled cadavers ion the morgue before coming to the maternity ward.
- Semmelweiss conducted experiments that hand washing would reduce the incidence of disease from 30% to less than 3%.
- Physicians still refused to wash their hands and admit that they were unclean

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Impressed with Pasteur's studies on the involvement of microorganisms in fermentation, **1867 Joseph Lister** demonstrated that boiling instruments and applying carbolic acid to dressings that covered wounds dramatically reduce the incidence of disease following surgery.

- Developed a system of antiseptic surgery designed to prevent microorganisms from entering the wounds.
- Instruments were heat sterilized (boiled)
- Phenol was used on surgical dressing and at time sprayed over the surgical area.
- This approach transformed surgery

NOW, **Robert Koch demonstrated the first direct role of a bacterium in disease**

In 1872 Koch began his work on the disease **called anthrax**, which is a devastating disease that affects cattle, often wiping out entire herds.

Anthrax is common disease but can also be transmitted to humans.

- In most common form of the disease, *B. anthracis* enters the body through skin abrasions,
- gets into the bloodstream,
- causes septicemia and death.

Koch established that a specific bacterium, ***Bacillus anthracis*** was the cause of the disease in mammals.

KOCH'S POSTULATES

In **1881 Koch proposed 4 postulates** that could be used to prove whether or not an infectious agent is the cause of a disease.

- I The causative agent must be present in every case of the disease and absent in healthy animals
- II The agent of disease can be isolated from the diseased animal and can be grown in pure culture (A population of one organism)
- III The disease can be reproduced by inoculating a portion of the pure culture into healthy animals
- IV The agent of disease can be re-isolated from the infected animal

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- Koch's proof that *Bacillus anthracis* caused anthrax was independently confirmed by Pasteur and his coworkers. They discovered that after burial of dead animals, anthrax spores survived and were brought to the surface by earthworms. Healthy animals then ingested the spores and became ill.

During the next thirty years 1880-1910 intense there was an competition between French and German bacteriologists as each country raced to identify as many causative agents of disease as possible.

This period of time is referred to as the ***Golden Age of Microbiology*** and the level of competition was analogous to the space race between the US and USSR

- Example of the modern relevance of Koch's postulates: Peter Duesberg's hypothesis that the human immunodeficiency virus (HIV) does not cause AIDS.
- Many bacterial agents of major human diseases were identified, and public health measures for disease prevention were devised and instituted.
- One important example: ***Koch's discovery of the tubercule bacillus*** (*Mycobacterium tuberculosis*) in 1882.

The advances made during the golden age would not have been possible without ***advances in cultivation techniques*** as well

Gelatin was routinely used as a solidifying agent for microbiological medium

Its use had numerous problems

- It would liquefy at high temperature
- It was often degraded by some bacteria

Fannie Hesse, the wife of one of Koch's Assistants Walter Hesse, suggested that they use Agar in place of gelatin

- Agar melts at 100° C, and would allow them to incubate cultures at body temperature (37 deg C)
- Agar is not attacked by bacteria

Richard Petri, also an associate of Koch developed the ***petri dish***, which allowed ***isolation of pure cultures***.

Koch also developed media that was suitable for growing bacteria isolated from the human body, many of which are still in use today

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Immunological Studies

During this golden age, advances were also made in discovering ***how animals resisted disease***.

During their studies on chicken cholera, Pasteur and Roux:

- Inadvertently discovered that prolonged incubation and subculture would attenuate the virulence of bacteria.
- ***Attenuate: that is they would lose the ability to cause disease***

If you injected the bacteria back into the animals they would not get sick, Moreover, they developed the ability to resist disease when challenged with a virulent organism

- Pasteur used this technique of attenuation to develop vaccines for Anthrax, Tetanus, and Rabies.

Shibasaburo (SHE BASA BURRO) Kitasato found that injecting inactivated diphtheria toxin into rabbits would cause them to produce a substance that would inactivate the toxin and protect against the disease.

This work with “Anti toxins” offered proof that there were soluble substances in the blood that were responsible for immunity (***Antibodies***) – ***humoral immunity***.

Eli Metchnikoff demonstrated that elements of the blood itself, leukocytes, was important to immunity (***cellular immunity***).

- He discovered that some cells were capable of engulfing disease causing bacteria. He called these leukocytes, Phagocytes (EATING/CELLS) and the engulfing process ***Phagocytosis***.

Advances in the field of microbiology have continued into the 20th century, and progress has been made at an incredible rate

Some of the more notable advances which will be covered in detail later this semester are

- discovery of antibiotics
- immunology and cancer research
- molecular biology and recombinant DNA technology

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Why is it important to study microorganisms?

Abundance	Negative impact on humans	Positive impact on Humans
	Disease	Biodegradation
	Food Spoilage	Food Production
	Biodegradation	Food Source
		Element Recycling
		Production of Industrial and Medical Products
		Genetically-engineered Microorganisms (GEMs)

Abundance

- Microbes are ubiquitous and prolific in the world around us.
- They inhabit the air we breath, the food and water that we eat and drink, the ground that we walk on, and our bodies!
- The surface of our skin contains over 2 million (2×10^6) microbes per square inch.
- A single gram of fecal material, contains over 100 billion (1×10^{11}) bacteria
- A human being consists of approx. 100 trillion (1×10^{14}) cells. Of that number only 10% are mammalian in origin. The remaining 90% are microbes and together weigh about one-quarter of a pound.
- Microbes are most common in soils, especially where there is a potential source of food.
- On average, one gram of soil harbors more than 10 million microbes (1×10^7)
- And when we count the number of microorganisms in the air that we breath, we find that it contains 50-100 microbes per cubic foot
- Microbes have developed some extraordinary survival adaptations that enable them to exist in a wide range of environments..

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- Many microorganisms form cysts or spores, when the food or water source disappears.
- The organisms can exist in this dormant state for years until the environment becomes more favorable
- As the nutrient source becomes more abundant, the spores can revive and develop into viable organisms.
- Archaeologists found, and successfully revived spores that that were dormant for thousands of years in sealed amphoras on greek shipwrecks.
- A couple of years ago, *Cano et al* reported that their laboratory successfully revived and cultivated spores contained in fossilized amber (Jurassic Bacteria).

In spite of their abundance, microorganisms are most noted for their negative impact on the human lifestyle.

In fact when microbes are most commonly associated with their ability to cause disease.

Disease is not the only way in which microbes have a **negative** impact on humans. They can also profoundly affect our lives through ***food spoilage and Biodegradation***.

We have all, at one time or another encountered:

- (a) Bread mold
- (b) Soft rot of fruits and vegetables
- (c) Soured milk
- (d) Canned food spoilage (botulism)
- (e) Putrefied meat

Examples of Biodegradation

- (a) Wood rot, rubber, paint, metal, cloth, etc.
There is not a single compound made by man which cannot be destroyed by a microorganism
- (b) Example:
book covers have to be varnished in a humid climate.

Believe it or not, Microbes can also have a ***Positive Impact*** on humans

Reference: *National Geographic, August 1993, pp. 36-61.*

Microbe mediated Biodegradation can be used to clean-up the environment
MEOR

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- Many fungi and genetically engineered bacteria can be used to effect a gradual breakdown of most toxic wastes, oil spills, pesticides, detergents, and other environmental pollutants.
- Microbes are a critical component of modern sewage treatment processes.
- If it weren't for microorganisms we would all be rotting in our own waste products

Food Production

Microbes can also be utilized in the production of Food

- Fraternity parties just wouldn't be the same without the fermentative metabolism of the various fungi used to make Alcoholic beverages (beer, wine, and liquors)
- Microbes are also responsible for Dairy products such as cheese, butter, and yogurt
- Yeast is used as a leavening agent for making bread
- And microbial fermentation reactions are also used to produce Vinegar

In some cases Microorganisms themselves, can be used as a food source

- Single-cell protein used as cattle feed (Pruteen)
- *Spirulina* (a bacterium) eaten as a health food.

Reference: Microbiological Reviews 47: 551-578 (1983).

Microbes are critical to ***Element recycling in the environment*** : the carbon, nitrogen, and sulfur cycles.

And Microorganisms are used in the production of ***numerous industrial and medical products***

- (a) Organic solvents (acetone, toluene)
- (b) Vitamins
- (c) Antibiotics
- (d) Vaccines
- (e) Even plastics!

* Genetically-engineered microorganisms can be used as biological factories to produce a wide array of biomedical reagents and as a source for useful genes (i.e. for gene therapy)

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In Summary

Over 90% of all known species of microorganisms are either neutral or beneficial to human beings. Less than 10% are harmful in some way.

Microbes do what they do independent of human values.
They are “good” or “bad” depending upon how we choose to see them.

One important goal of microbiology is to better understand the activities of these organisms so that we can minimize what we consider to be their harmful effects and maximize their beneficial effects.

Questions we will address in this course

- (1) What are microorganisms?
- (2) Where are they found?
- (3) How do we identify them?
- (4) What activities do they have?
- (5) How do they affect us?
- (6) How can we control them?