

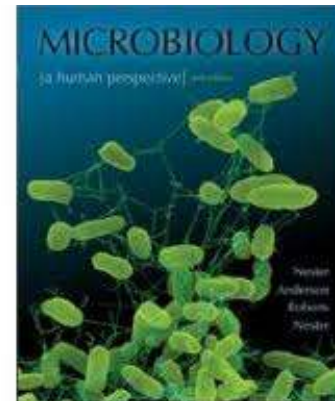
# Microorganism & Microbiology

## Microorganism

- Living things which individually are too small to be seen with the naked eye.
- All of the following may be considered microorganisms:
  - bacteria (eubacteria, archaeobacteria)
  - fungi (yeasts, molds)
  - protozoa
  - microscopic algae
  - viruses
  - various parasitic worms

## Microbiology

- Study of microorganisms
- Foundation of modern biotechnology
- Among the many specialized fields of microbiology
  - Virology, Mycology, Bacteriology, Immunology, Microbial Ecology, Biotechnological Microbiology, Environmental Microbiology, Food Microbiology, Forensic Microbiology, Molecular Biology

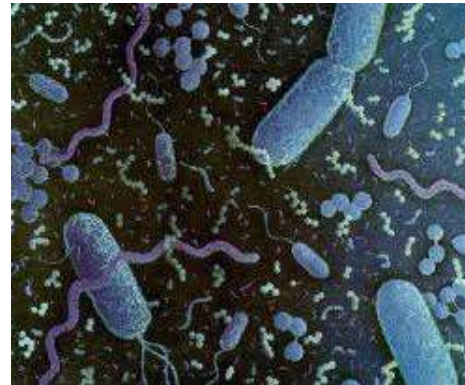
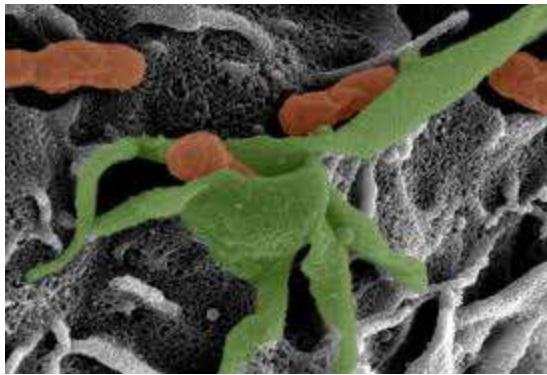


# Microorganism & Microbiology cont'd

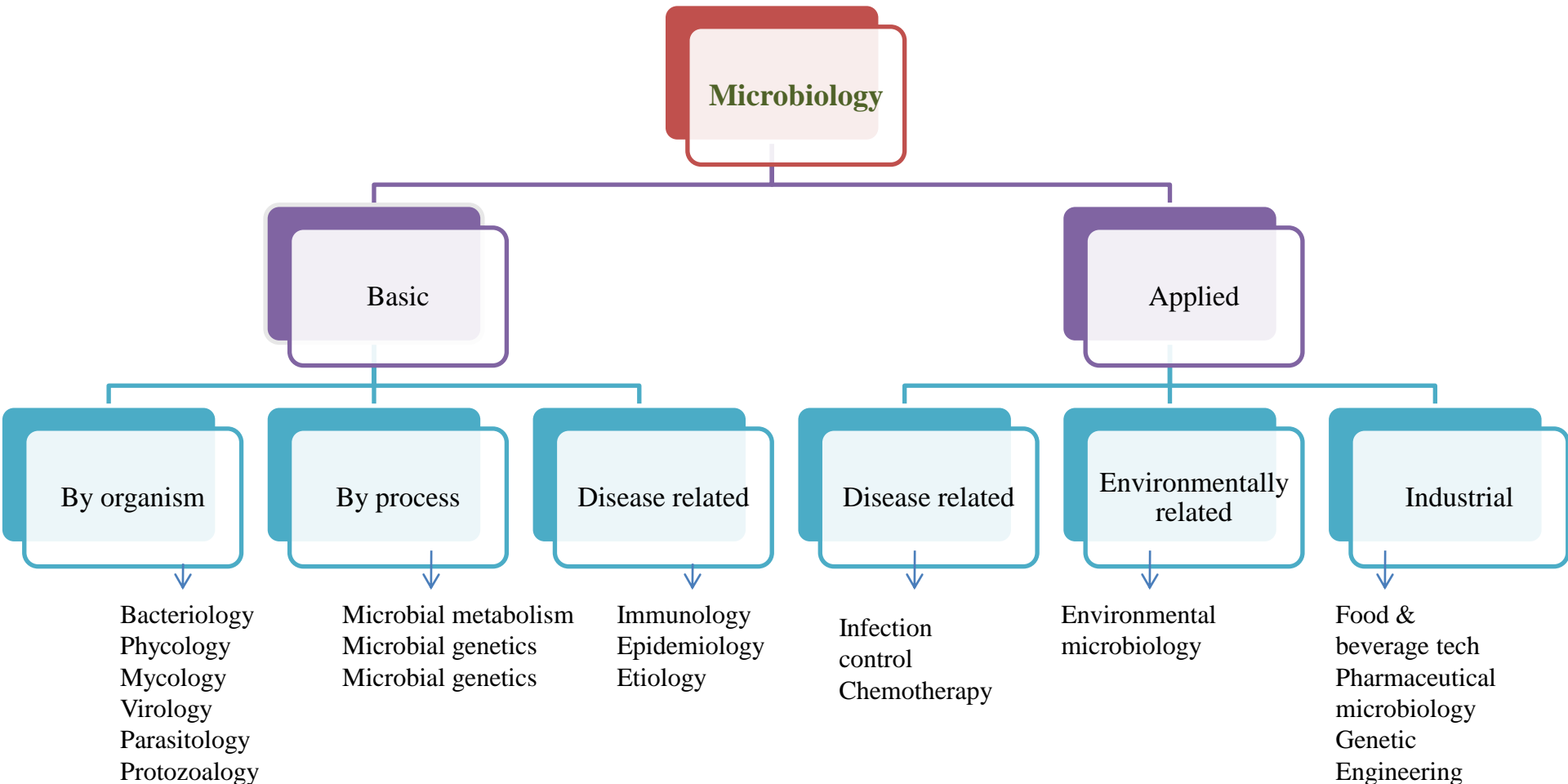
Two main themes involved in Microbiology

1- **Basic**- cellular processes

2- **Applied**- concerning agriculture, industry and health



# Themes in Microbiology and its field



# Microorganism

- Too small
- Germ-rapidly growing cell
- Has habitat
- Live in population (not alone)
- Communities are either swimming freely or attached to a surface (biofilm)
- Interact between communities; may either be
  - harmful (because of waste product)
  - beneficial (cooperative feeding efforts-waste → nutrient)



# Microbes in our lives

- Some are pathogenic (disease-causing)
- Decompose organic waste
- Produces through photosynthesis (e.g. Purple sulphur bacteria must fix CO<sub>2</sub> to live)
- Play role in industry (e.g. fermentation to produce ethanol and acetone)
- Produce fermented food (vinegar, cheese & bread)
- Produce products used in manufacturing (cellulase) and treatment (insulin)



# Microbes in our lives



## Food

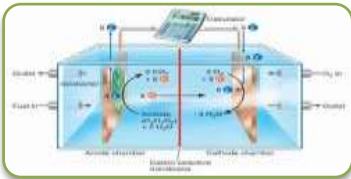
- Lactococci
- Ferment milk



## Water treatment

Such as certain bacteria belonging to the *Bacillus* species

1. Digest a wide variety of organic material that are present in wastes.
2. Digest waste quickly and completely, without producing significant odors of noxious gas.
3. Non-pathogenic.
4. Grow and reproduce quickly and readily in the environmental conditions found in waste disposal systems.



## Energy

- Produce methane



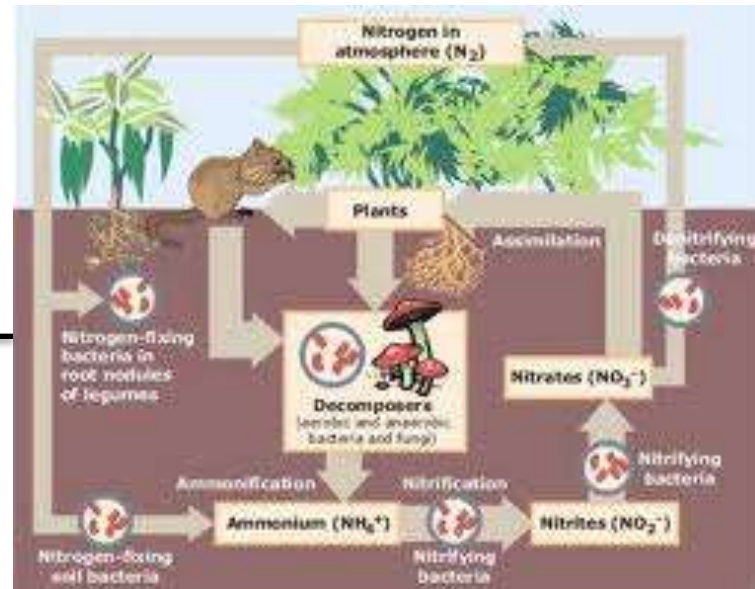
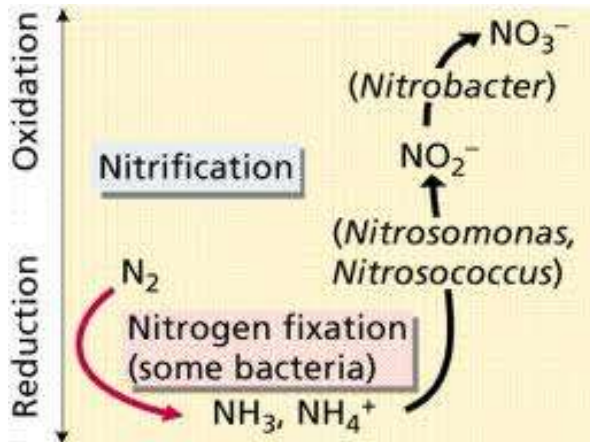
## Science- laboratories



## Warfare -anthrax

# Microbes and agriculture

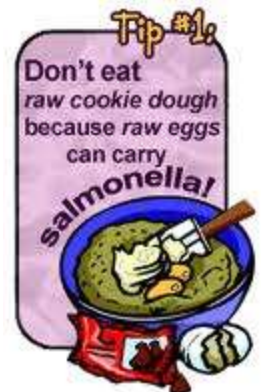
- Nitrogen fixation



- Rumen microbes help digest grass and hay in cows, sheep etc
- Cycles nutrients (C, N and S)
- Causes disease to animals and plants

# Microorganism and Food

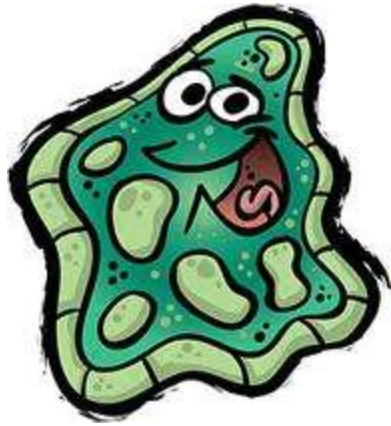
- Microorganism and food
  - 1) Prevent spoilage (tempeh, salted fish)
  - 2) Assist in manufacturing of food
- Microorganisms and energy
  - 1) Natural gas (methane)
  - 2) Ethanol (biofuel)
  - 3) Bioremediation
- Microbes and the future
  - 1) Genetic engineering





# Microbes and diseases



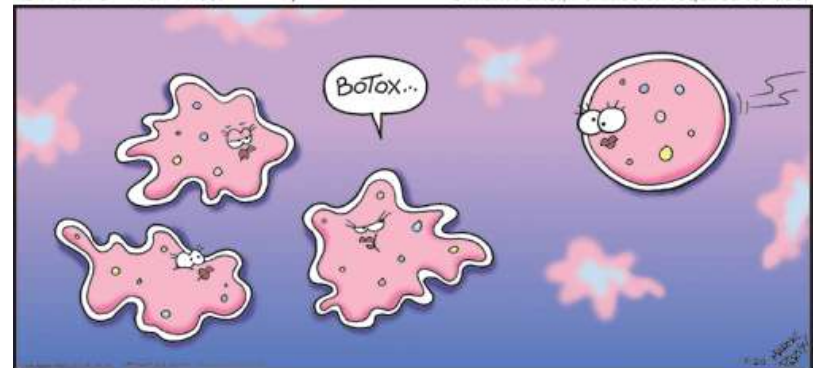


# Thoughts for the day

So, how can microbes benefit us?

- In food?
- In environment?
- In preventing disease?
- In agriculture?
- In energy?
- In waste-water treatment?

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# Naming and Classifying microorganisms

- Linnaeus system for scientific nomenclature
- Each organism has two names:
  - 1) Genus
  - 2) Specific epithet

# Scientific Names

- Italicized or underlined.

The genus is capitalized, and the specific epithet is with lowercase

- Could be as an honor for the scientist

- A Latin origin

e.g. *Escherichia coli* (*E. coli*)

- discoverer: Theodor Escherich
- describes the habitat (colon/intestine)



In intestine

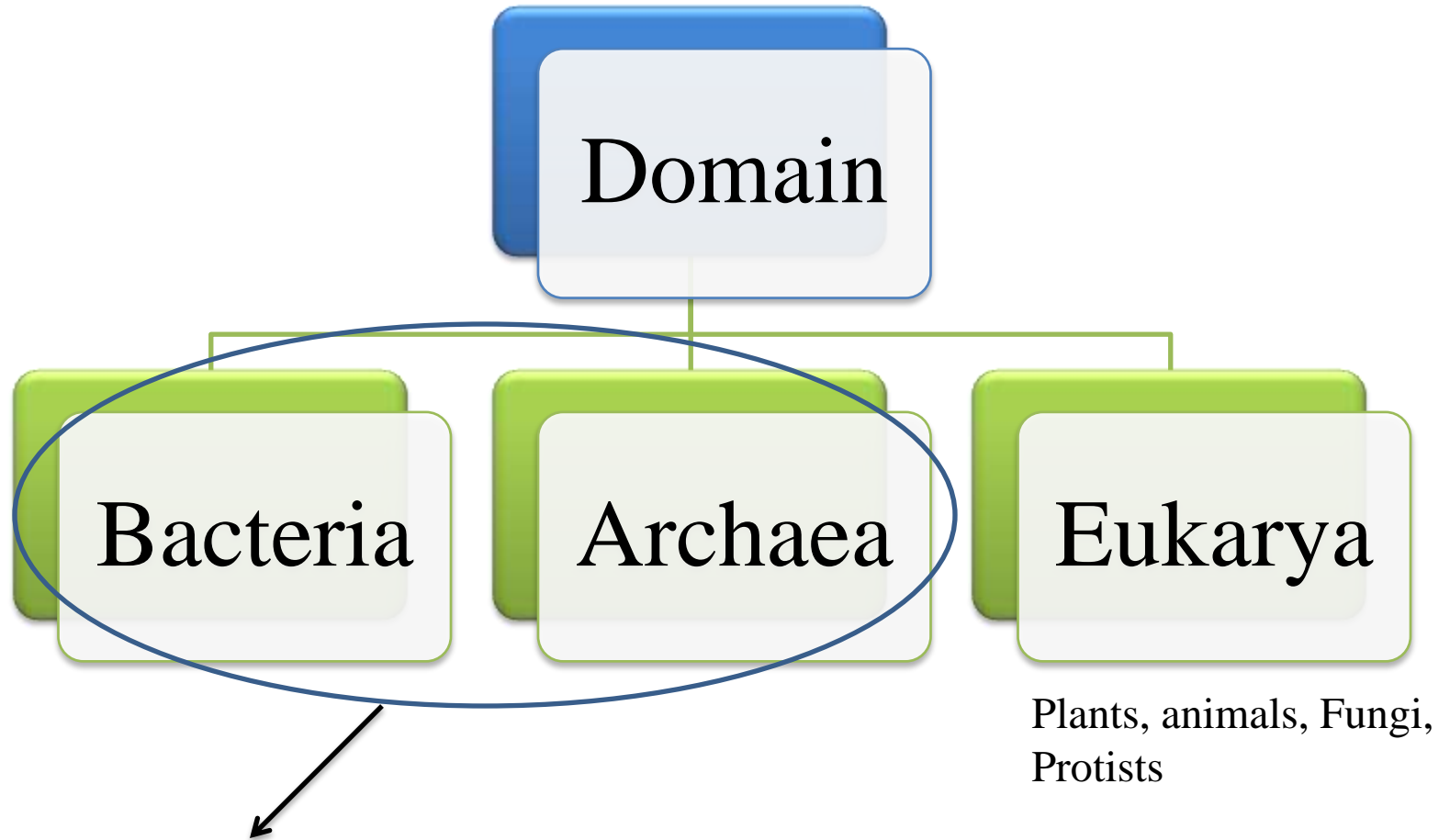
e.g. *Staphylococcus aureus* (*S. aureus*)

- Clustered (staphylo), spherical (cocci)
- Gold colored colonies (aureus)



On skin

# Classification of bacteria



**Microorganisms**

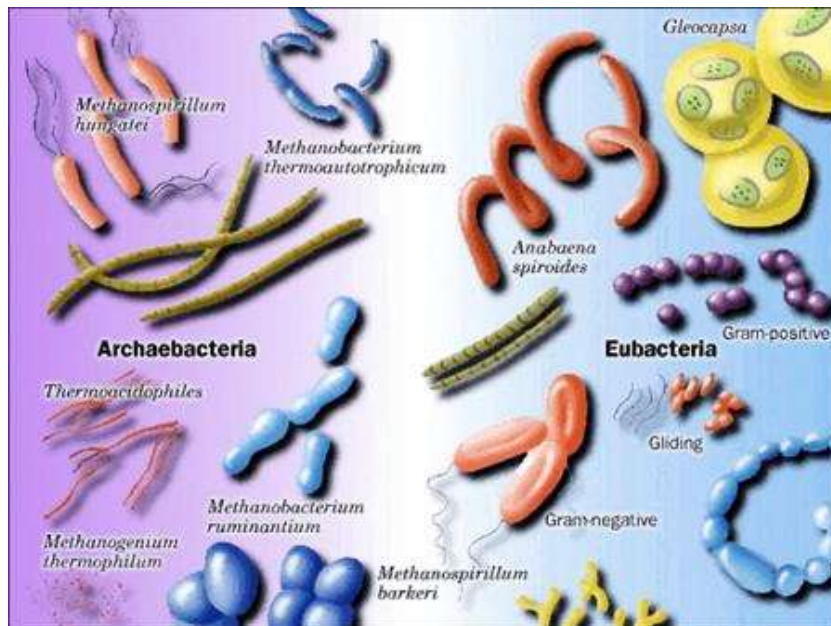
Also include  
fungi, protozoa, algae, viruses, multicellu  
lar animal parasites

## Bacteria (P)/ Bacterium (S)

- Prokaryotes
- Has peptidoglycan cell walls
- Binary fission
- Utilize organic/inorganic chemicals, or photosynthesis to obtain energy

## Archaea

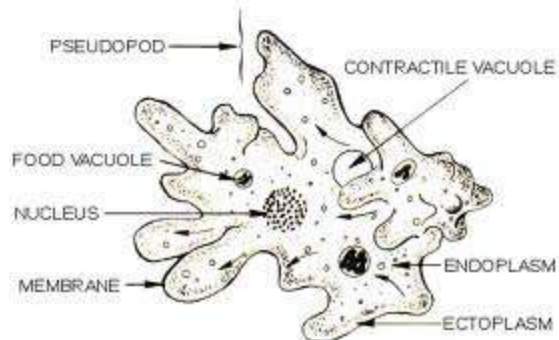
- Prokaryotic
- Lack peptidoglycan
- Live in extreme environments
- Include
  - Methanogens
  - Extreme halophiles
  - Extreme thermophiles



# Types of Eukaryotes

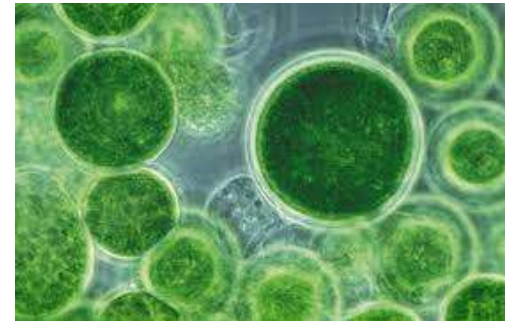
## Protozoa

- Unicellular eukaryote
- Absorb or ingest organic chemicals
- May move using pseudopods, cilia or flagella
- e.g. Amoeba



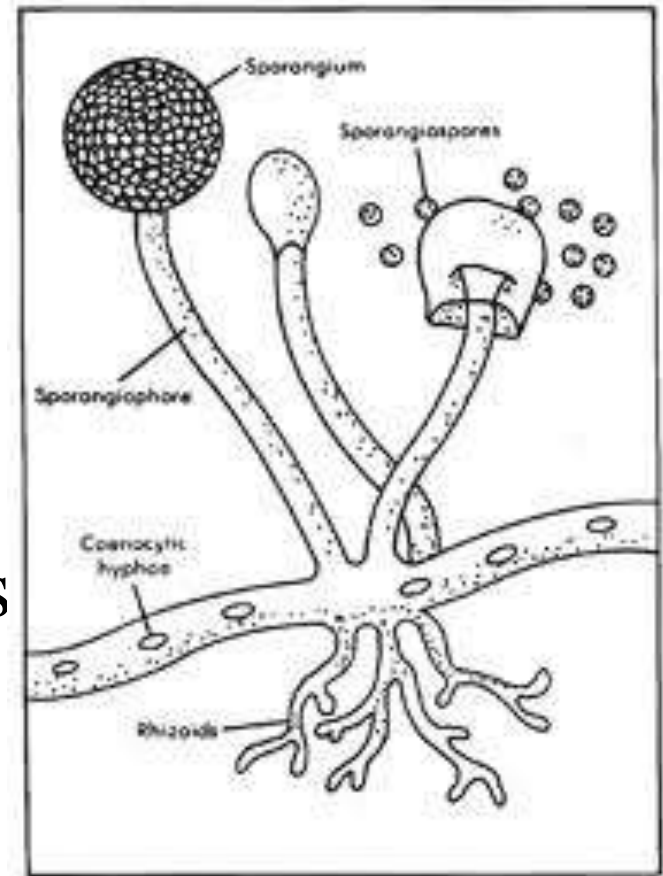
## Algae

- Unicellular/multicellular eukaryote
- Has cellulose cell walls
- Gain energy through photosynthesis
- Produce molecular and organic compounds



# Fungi (singular: Fungus)

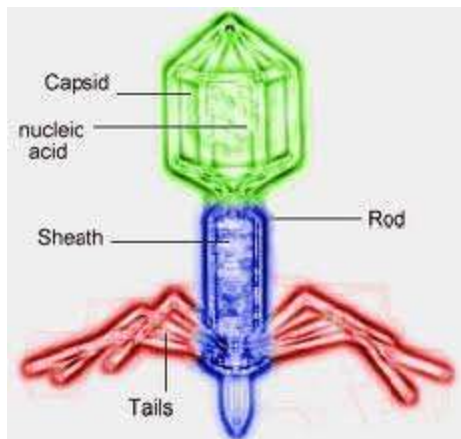
- Eukaryotes
- Chitin cell walls
- Use organic chemicals for energy
- Molds and mushrooms are multicellular, consists of mycelia (composed of filaments called hyphae)
- Yeasts are unicellular





# Viruses

- Too small to be observed with light microscope
- Consists of DNA/RNA core
- Core is surrounded by protein coat
- Coat may be enclosed in a lipid envelope
- Viruses are replicated only when they are in living host cell
- Bacteriophage-viruses that infect bacteria
- Viroids-nucleic acid without protein coating
- Prions- Infectious proteinaceous particles



# Multicellular animal parasites

- Helminths: flatworms and roundworms
- Multicellular



# How do we view microorganisms?

- Units of measurement

When talking about cells and microscopic organisms, you would be measuring using **MICROMETRE** (abbreviated:  $\mu$  --micron ) or stated as:  $\mu\text{m}$  (micrometer).

$$1 \mu\text{m} = 1 \times 10^{-6} \text{ meters} / 1 \times 10^{-3} \text{ mm}$$

$$1 \text{ mm} = 1 \times 10^3 \text{ nanometers} / 1 \times 10^3 \mu\text{m}$$

To give you the idea of how small a micro metre is,

1- a human hair is about 100  $\mu\text{m}$ , wide,

2- a red blood cell would be around 8  $\mu\text{m}$  wide

3- typical size of an animal cell would be from 10 - 100  $\mu\text{m}$

# Microscope

Light microscope

- Uses light
- Few types

Compound light microscopy

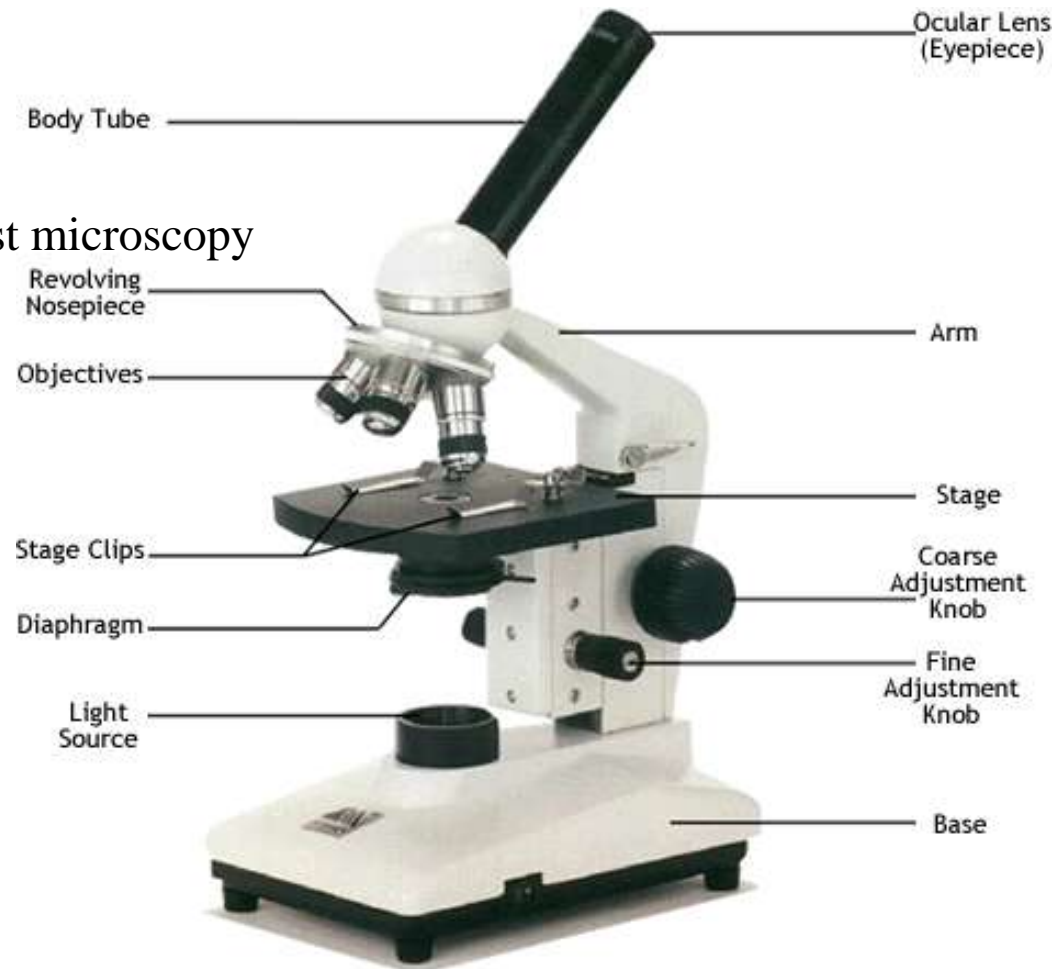
Darkfield microscopy

Phase-contrast microscopy

Differential interference contrast microscopy

Fluorescence microscopy

Confocal microscopy

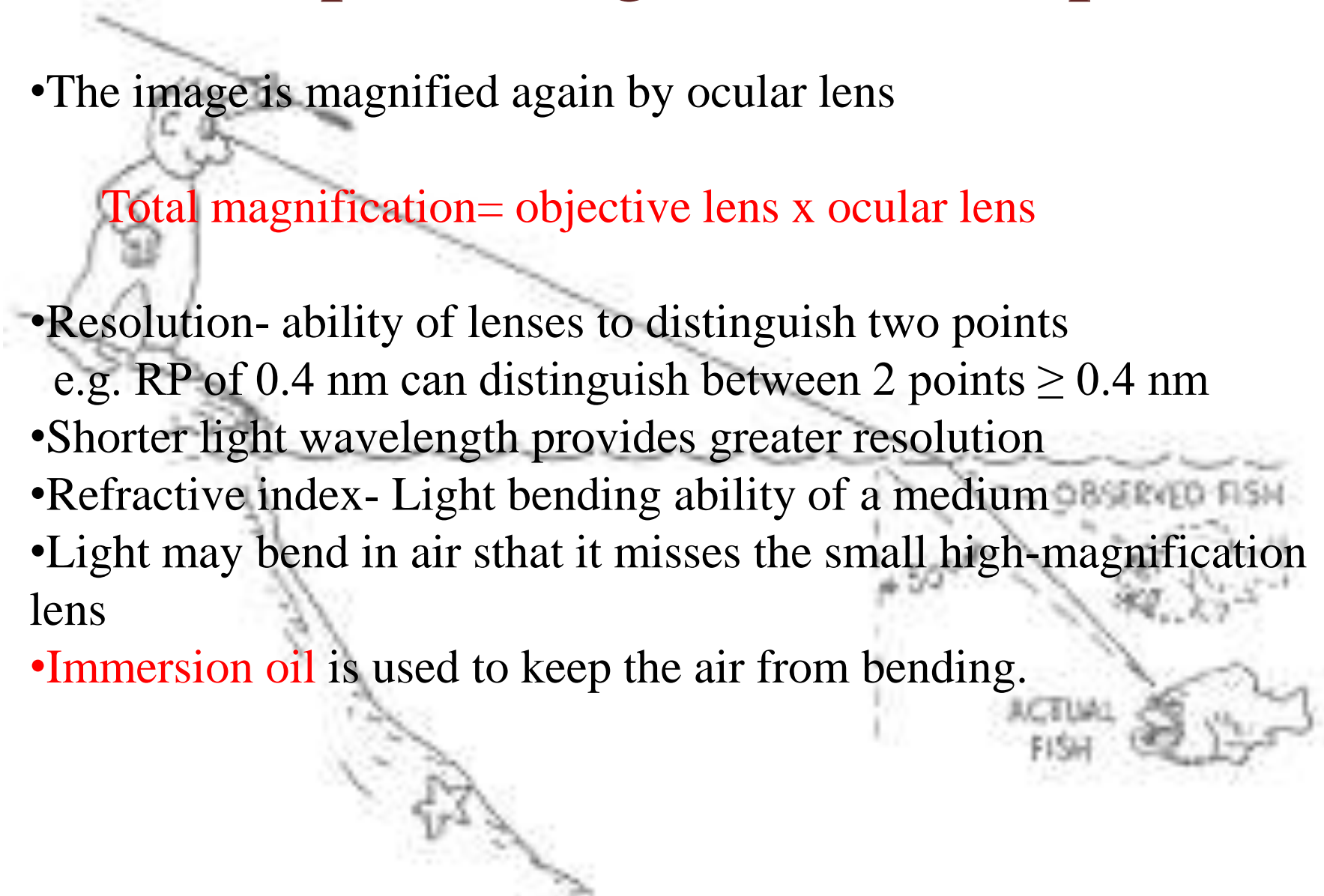


# Compound light microscope

- The image is magnified again by ocular lens

**Total magnification = objective lens x ocular lens**

- Resolution- ability of lenses to distinguish two points  
e.g. RP of 0.4 nm can distinguish between 2 points  $\geq 0.4$  nm
- Shorter light wavelength provides greater resolution
- Refractive index- Light bending ability of a medium
- Light may bend in air sthat it misses the small high-magnification lens
- **Immersion oil** is used to keep the air from bending.



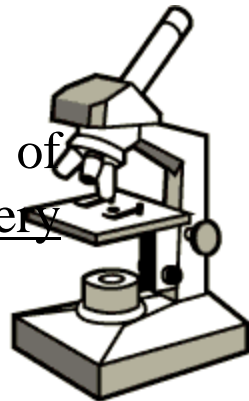
# Types of Microscopes

**Light Microscope** - found in most schools, use compound lenses and light to magnify objects. The lenses bend or refract the light, which makes the object beneath them appear closer.

**Stereoscope** - this microscope allows for binocular (two eyes) viewing of larger specimens. (The spinning microscope at the top of this page is a stereoscope)

**Scanning Electron Microscope** - allow scientists to view a universe too small to be seen with a light microscope. SEMs do not use light waves; they use electrons (negatively charged electrical particles) to magnify objects up to two million times.

**Transmission Electron Microscope** - also uses electrons, but instead of scanning the surface (as with SEM's) electrons are passed through very thin specimens. Specimens may be stained with heavy metal salts



# Parts of the Microscope

Parts	Functions
Eyepiece	To observe specimen. Contains two or more lenses. The most common magnification for the eyepiece is 10X. There are also 2x and 5x. An eye piece is a removable, can be interchanged for different magnification.
Objective Lenses	More than one objective lenses. These are the primary lenses of a compound microscope and can have magnification of 4x, 5x, 10x, 20x, 40x, 50x and 100x.
Stage	The platform below the objective lens on which the object to be viewed is placed. A hole in the stage allows light beam to pass and illuminate the specimen.
Stage Clips	There are two stage clips one on each side of the stage. Once the slide containing the specimen is placed on the stage, the stage clips are used to hold the slide in place.
Diaphragm	It is located on the lower surface of the stage. It is used to control the amount of light that reaches the specimen through the hole in the stage.
Illuminator	Simple compound microscopes have a mirror that can be moved to adjust the amount of light that can be focused on the specimen. However, some advanced types of compound microscopes have their own light source.
The Adjustments	2 adjustment knobs: fine adjustment & coarse adjustment knob; refine the focus of the lenses. The coarse adjustment knob helps in improving the focus of the low powers whereas the fine adjustment knob helps in adjusting the focus of the lenses with higher magnification.

# Magnification

Your microscope has 3 magnifications:

**Scanning**, **Low** and **High**. Each objective will have written the magnification. In addition to this, the ocular lens (eyepiece) has a magnification.

The total magnification is the ocular x objective.

Total magnification = magnification of eyepiece x magnification of objective lens

	Magnification	Ocular lens	Total Magnification
Scanning	4x	10x	40x
Low Power	10x	10x	100x
High Power	40x	10x	400x

# A Brief History of Microbiology

## Development of microscopy

- (384-322) **Aristotle** and others believed that living organisms could develop from non-living materials.
- 1590: **Hans and Zacharias Janssen** (Dutch lens grinders) mounted two lenses in a tube to produce the first compound microscope.
- 1660: **Robert Hooke** (1635-1703) published "Micrographia"; drawings and detailed observations of biological materials made with the best compound microscope and illumination system of the time.
- 1676: **Anton van Leeuwenhoek** (1632-1723) 1<sup>st</sup> person to observe microorganisms.
- 1883: **Carl Zeiss** and **Ernst Abbe** pioneered developments in microscopy (such as immersion lenses and apochromatic lenses which reduce chromatic aberration) exist until the present day.
- 1931: **Ernst Ruska** constructed the 1<sup>st</sup> electron microscope.



# Van Leeuwenhoek's description of Bacteria

From his teeth, he observed

(A) & (B)- rod forms

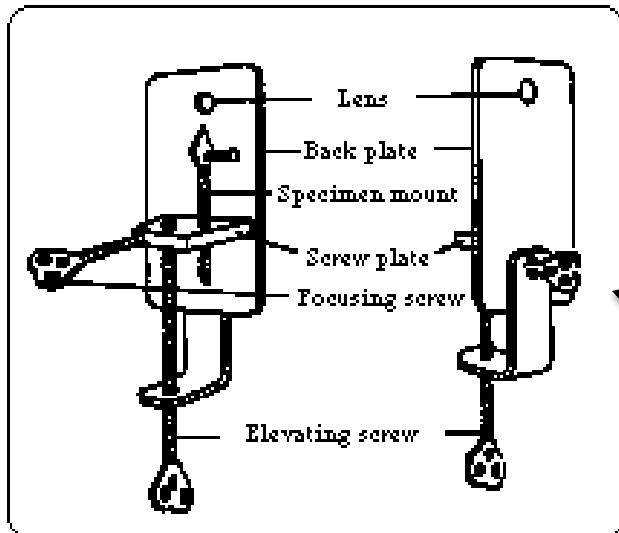
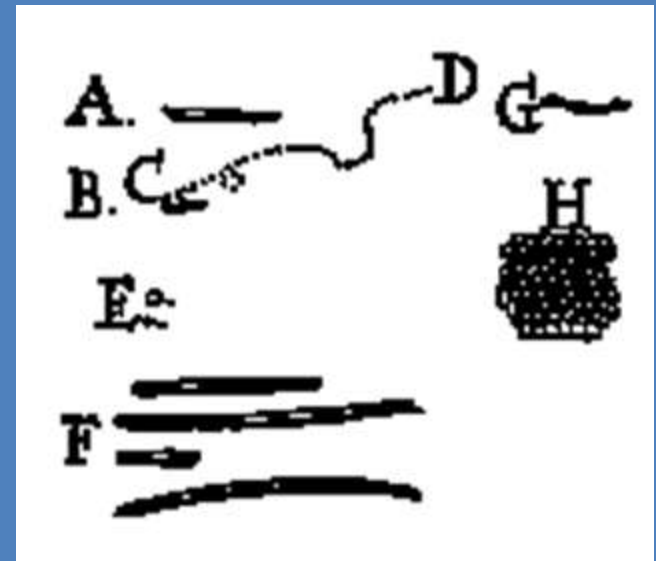
(C) & (D)- motion pathway

(E)- Spherical form

(F)- Longer type of spherical form

(H)- Cluster

-Royal Society letter (Sept 17<sup>th</sup>, 1683)



The microscope used  
Simple microscope (one lens)

# Spontaneous generation controversy

1688: **Francesco Redi** (1626-1678) was an Italian physician who refuted the idea of spontaneous generation by showing that rotting meat carefully kept from flies will not spontaneously produce maggots.

1836: **Theodor Schwann** (1810-1882) helped develop the cell theory of living organisms, namely that that all living organisms are composed of one or more cells and that the cell is the basic functional unit of living organisms.

1861: **Louis Pasteur's** (1822-1895) famous experiments with swan-necked flasks finally proved that microorganisms do not arise by spontaneous generation.

# The Golden Age of Microbiology

~1857-1914 (about 50 years)

Beginning with Pasteur's work, discoveries included relationship between microbes and disease, immunity, and antimicrobial drugs

- Robert Koch**
- a. Identified a bacterium as cause of anthrax
  - b. Introduced agar, inoculating loop to transfer bacteria and prepare pure cultures.
  - c. Introduced "Koch's Postulates" and the concept that a disease is caused by a single organism.

**Joseph Lister** (1865)

- a. Introduced the "antiseptic technique".
- b. Use of phenol (carbolic acid) as disinfectant.

**Martinus Beijerinck** (1884 - 85)

- a. Discovered "viruses" (toxins, poisons).
- b. Infectious agents in tobacco plant fluids.

**Paul Ehrlich** (1910)

- a. Introduced concept of chemotherapy.
- b. Use of salvarsan for the treatment of syphilis.

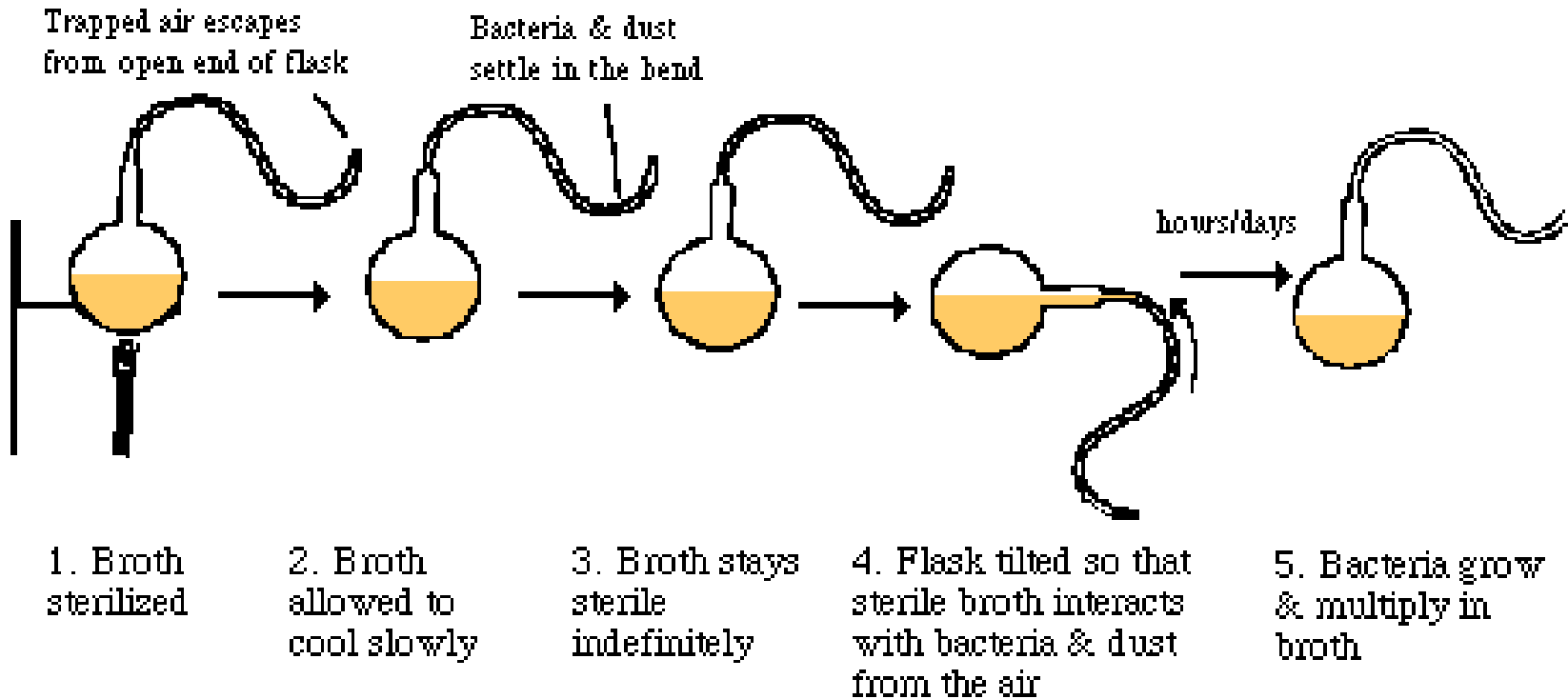
**Alexander Fleming** (1928)

- a. Discovered the first antibiotic - penicillin.

Louis Pasteur's (1822-1895) famous experiments with swan-necked flasks

This eventually led to:

- Development of sterilization
- Development of aseptic technique



# Proof that microbes cause disease

1546: **Hieronymus Fracastorius** (Girolamo Fracastoro) wrote "On Contagion", the 1<sup>st</sup> known discussion of the phenomenon of contagious infection.

1835: **Agostino Bassi de Lodi** showed that a disease affecting silkworms was caused by a fungus - the first microorganism to be recognized as a contagious agent of animal disease.

1847: **Ignaz Semmelweiss** (1818-1865), a Hungarian physician- decided that doctors in Vienna hospitals were spreading childbed fever while delivering babies. He started forcing doctors under his supervision to wash their hands before touching patients.

1857: **Louis Pasteur** proposed the "Germ theory of disease".

- Ancients believed that disease was the result of a divine punishment. Pasteur fought to convince surgeons that germs existed and carried diseases, and dirty instruments and hands spread germs and therefore disease. Pasteur's pasteurization process killed germs and prevented the spread of disease.

1867: **Joseph Lister** (1827-1912) introduced antiseptics in surgery. By spraying carbolic acid on surgical instruments, wounds and dressings, he reduced surgical mortality due to bacterial infection considerably.

1876: **Robert Koch** (1843-1910). German bacteriologist was the first to cultivate anthrax bacteria outside the body using blood serum at body temperature.

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

## Koch's postulates

"**Koch's postulates**" (1884), the critical test for the involvement of a microorganism in a disease:

1. The agent must be present in every case of the disease.
2. The agent must be isolated and cultured in vitro.
3. The disease must be reproduced when a pure culture of the agent is inoculated into a susceptible host.
4. The agent must be recoverable from the experimentally-infected host.

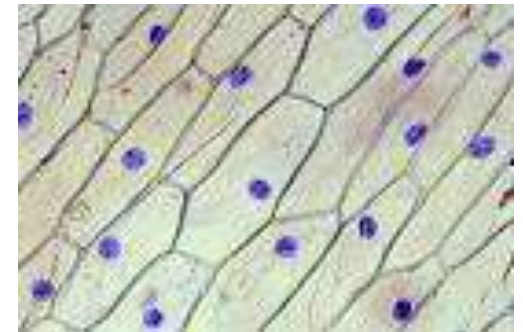
This eventually led to:

- Development of pure culture techniques
- Stains, agar, culture media, petri dishes



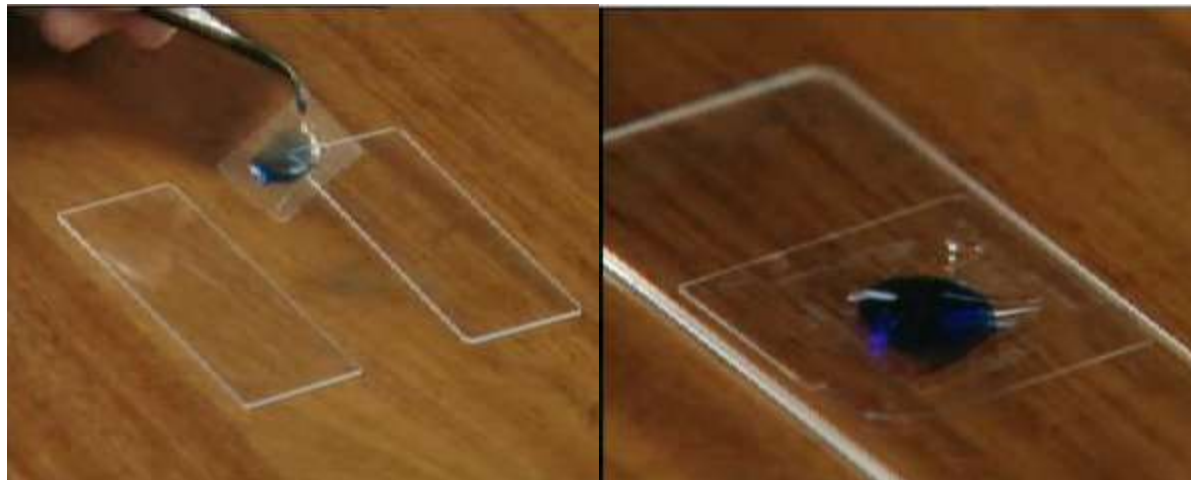
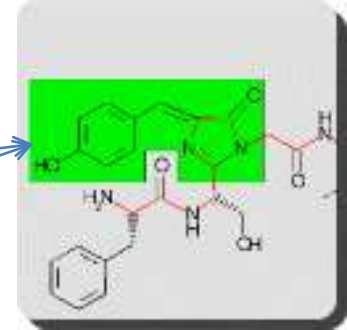
# Preparing smears for staining

- Staining- coloring microbe with a dye to emphasize certain structure
- Smear- A thin film of a microbe solution on a slide, a smear is usually fixed to attach microbes to the slide and kill microbes



# Staining

- Stain usually consists of +ve and -ve ion
- Basic dye- chromophore is a cation
- Acidic dye- chromophore is an anion
- Staining the background instead of the cell is called negative staining





# Staining

## Simple stain

- staining with one dye
- mordant may be used to hold the stain or to coat the specimen to enlarge it

## Gram stain

### Distinguish

- Gram +ve and gram -ve
- Gram +ve bacteria are prone to penicillin and detergents
- Gram -ve are more resistant to antibiotics

## Differential stain

### Distinguish

- Gram stain
- Acid-fast stain

## Acid-fast stain

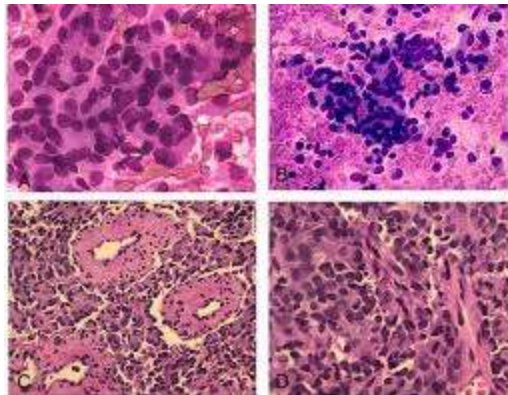
Stained waxy cell wall is not decolorized by acid-alcohol

- Mycobacterium
- Nocardia

## Special stain

Distinguish special parts of cells

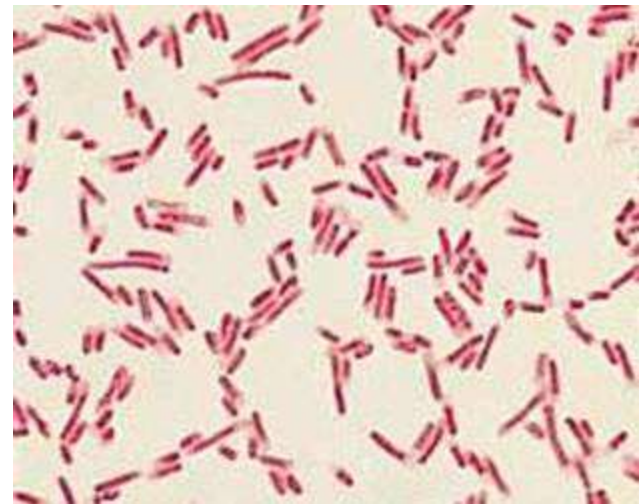
- Capsule
- Endospore (Malachite green and safranin)
- Flagella (carbofuchsin simple stain)



## Gram stain

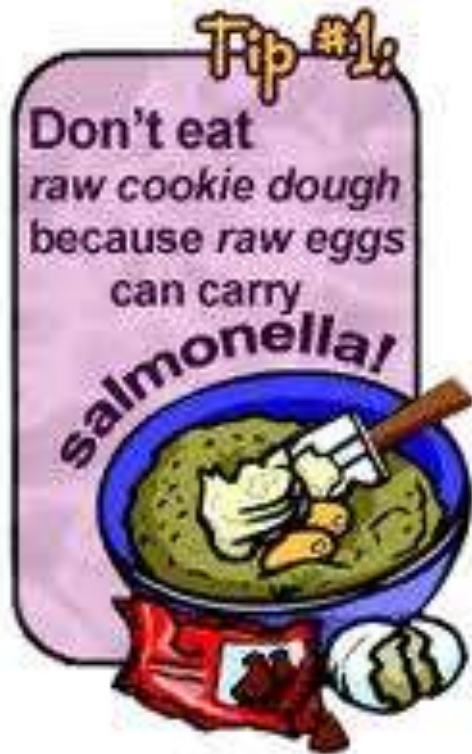
Distinguish

- Gram +ve and gram -ve
- Gram +ve bacteria are prone to penicillin and detergents
- Gram -ve are more resistant to antibiotics

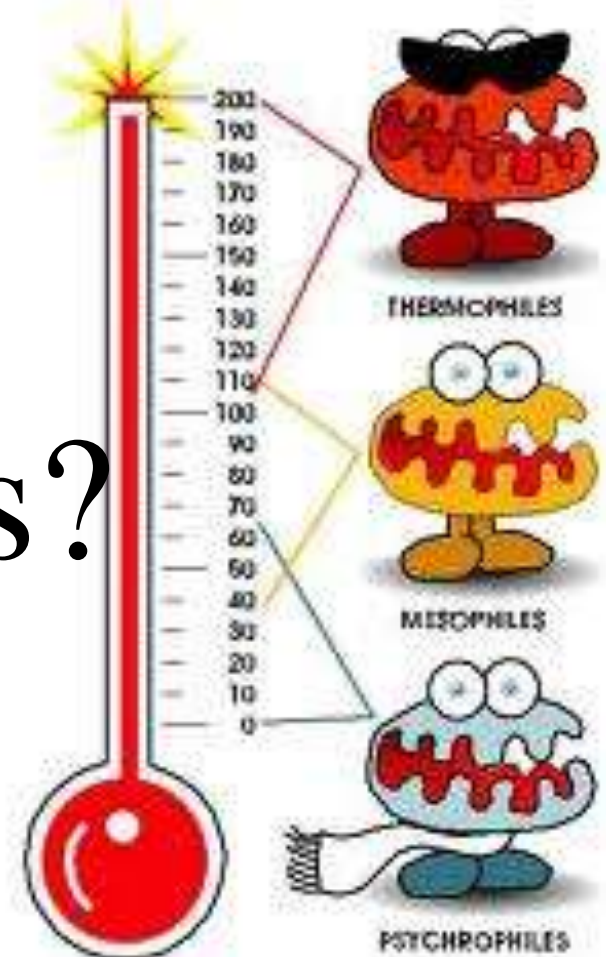


Gram -ve (pink)

So, microorganisms are something studied using characteristic techniques including:  
aseptic technique  
pure culture technique  
microscopic observation of whole organisms



# Questions?



**Recommended reading:**

**Microbiology**

by L.M.Prescott et al. 6<sup>th</sup> edition.