

# Environmental Microbiology

## The Microbial World

prepared by Prof. Bulent Içgen

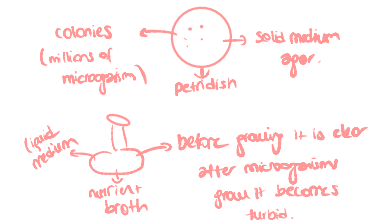
# Microbes

most of the microorganisms are not pathogenic  
↳ means can cause dises.

- Microorganisms are organisms that are too small to be seen with the unaided eye
- They are not all **GERMS!**
- **GERM** refers to a rapidly growing cell that can cause disease



# Microorganisms:



## Good

- Decompose organic waste (use them as a source of nutrients such as C, N, P) to grow.
- Are producers in the ecosystem by photosynthesis (Ex: cyanobacteria (some call blue-green algae), green-purple sulfuric bacteria, algae / phytoanktions)
- Produce industrial chemicals such as ethyl alcohol and acetone
- Produce fermented foods such as vinegar, cheese, and bread

mineral cycle depends on microorganisms.  
↳ without them no life.

## Bad

- Pathogenic → they have capsule  
smooth  
↓  
S type colony  
↳ if they are rough mostly non pathogenic.

# Naming & Classifying Microorganisms

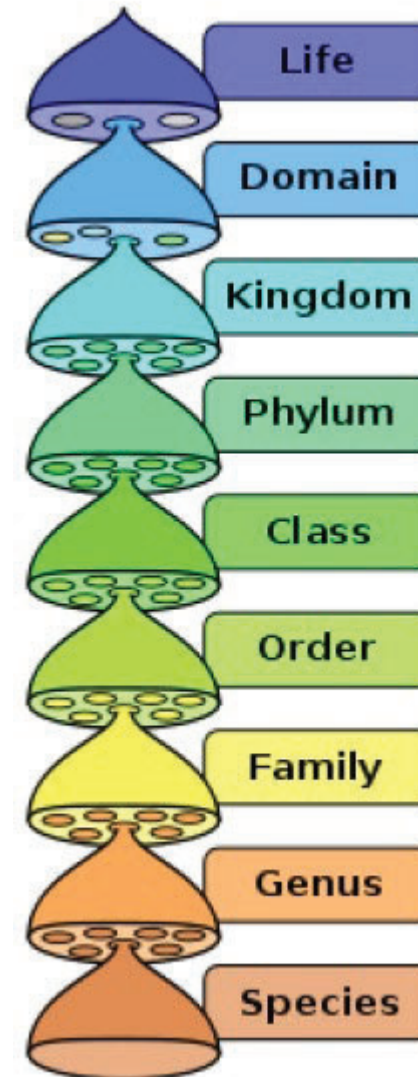
- The basic taxonomic unit is the *species*
- Microorganisms are generally given a genus and species name

*Escherichia coli*  
Genus Species

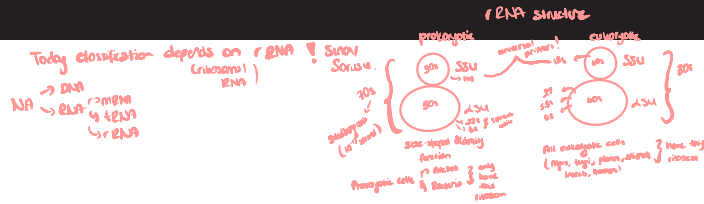
## Scientific name (Systematic Name) Binomial System of Nomenclature

- Genus name + species name  
Capital letter little letter
- Italicized or underlined → *must!*
- Genus name is capitalized and may be abbreviated
- Species name is never abbreviated

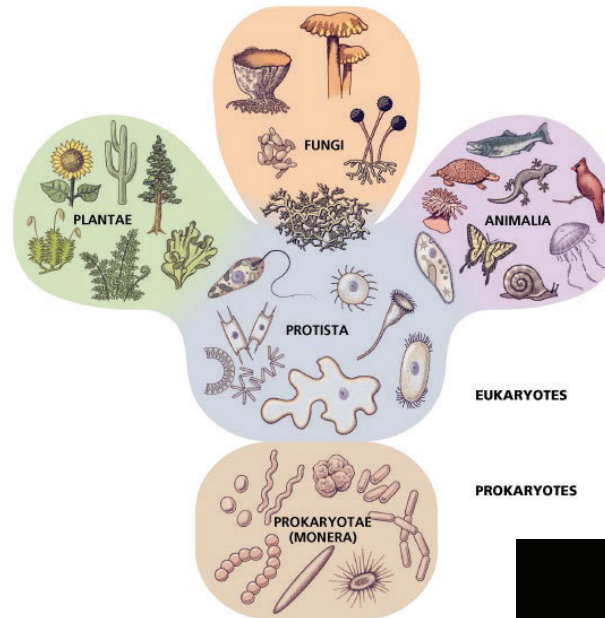
eg: *Bacillus subtilis*  
After first time you can abbreviate  
*B. subtilis*



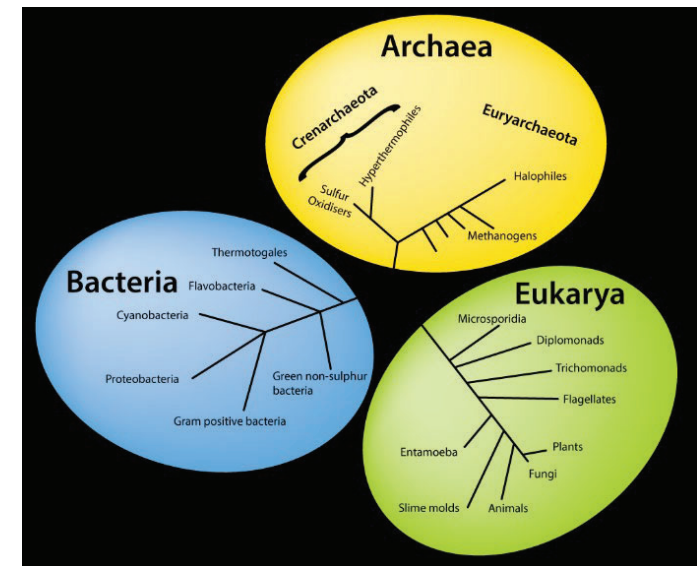
# Classification of Microorganisms



- Three domains
  - Bacteria
  - Archaea
  - Eukarya
    - Protists
    - Fungi
    - Plants
    - Animals



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# Microorganisms:

## Eukaryotic vs. Prokaryotic Cells

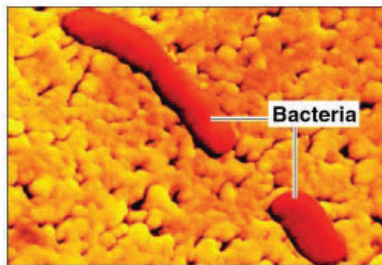
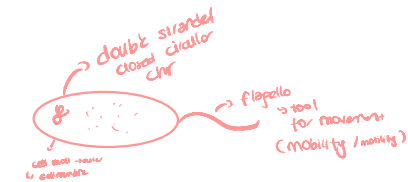
### ➤ Eukaryotes

- DNA enclosed in a membrane-bound nucleus
- Cells are generally larger and more complex
- Contain organelles

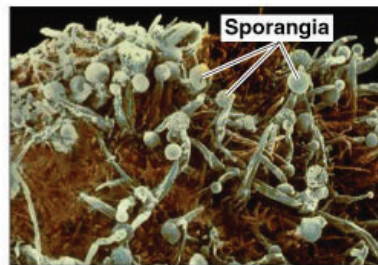


### ➤ Prokaryotes

- No membrane-enclosed organelles, no nucleus
- Generally smaller than eukaryotic cells



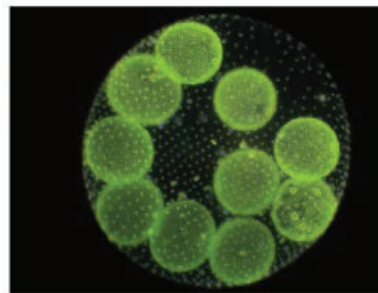
(a)



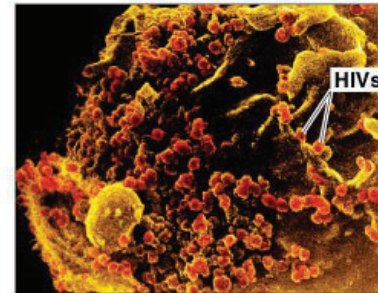
(b)



(c)

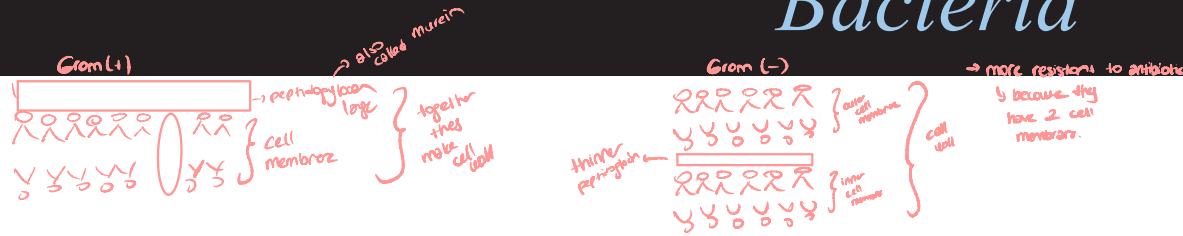


(d)

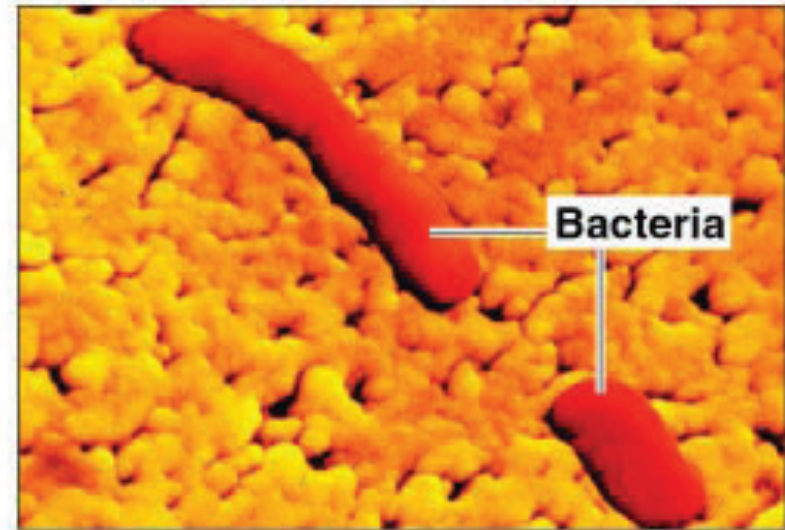


(e)

# Bacteria



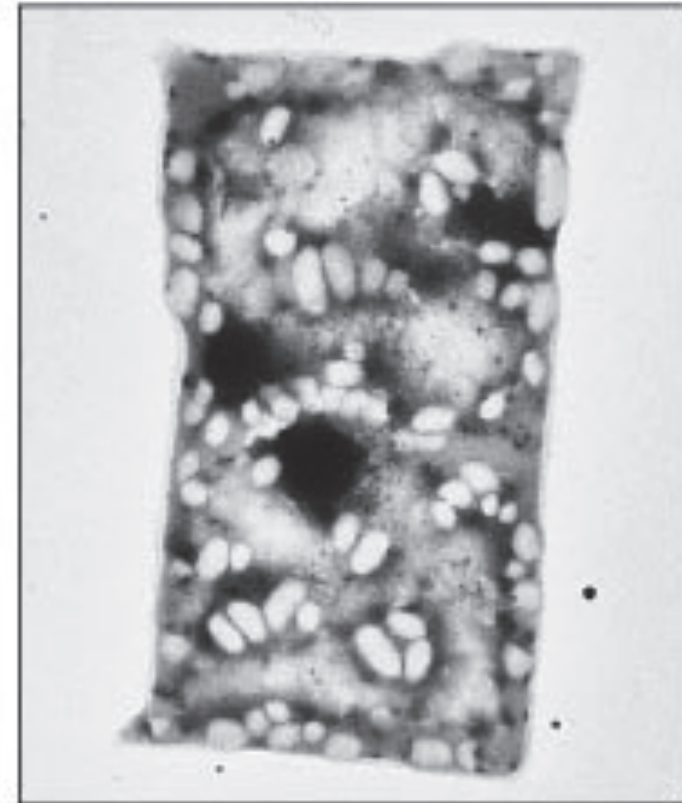
- Prokaryotes
- Peptidoglycan cell walls can cross microorganisms by cell walls
- Binary fission
- For energy, use organic chemicals, inorganic chemicals, or photosynthesis



(a)

# Archaea

- Prokaryotic
- Lack peptidoglycan (murein) → they have pseudo-murein
- Live in extreme environments
- Include:
  - Methanogens (they produce CH<sub>4</sub> in biogas)
  - Extreme halophiles (high salt (NaCl) → osmotic concentrations)
  - Extreme thermophiles

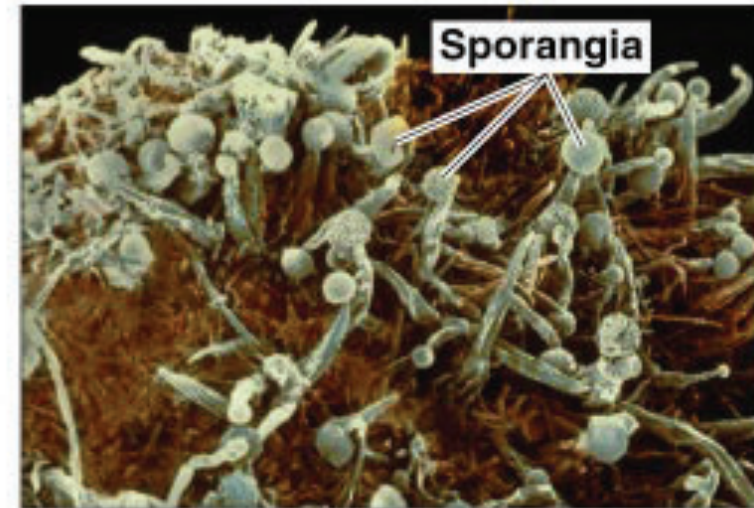


# Fungi

- Eukaryotes
- Chitin cell walls
- Use organic chemicals for energy
- Molds and mushrooms are multicellular consisting of masses of **mycēlia**, which are composed of filaments called **hyphae**
- Yeasts are unicellular

Saccharomyces cerevisiae (Brewer's Yeast)  
S. cerevisiae → yeast

↳ ner bi flonra-ti kofun



(b)

# Protozoa

- Eukaryotes
- Absorb or ingest organic chemicals
- May be motile via **pseudopods**, **cilia**, or **flagella**

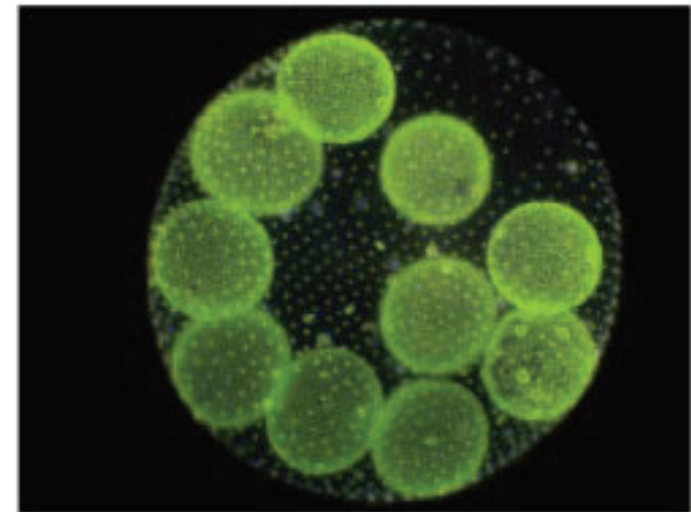
Ameoba



(c)

# Algae

- Eukaryotes
- Cellulose cell walls → they look like plants
- Use photosynthesis for energy
- Produce molecular oxygen and organic compounds



(d)

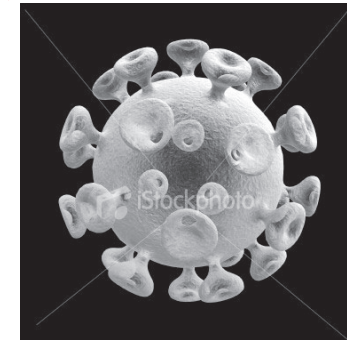
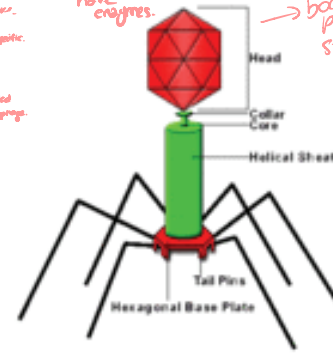
# Viruses

they need a host to survive → because they don't have enzymes.  
 (it is a mist)  
 ↳ without host → stay on rot alive.

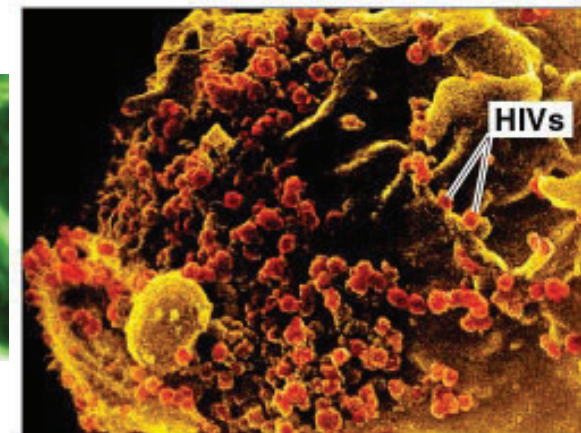
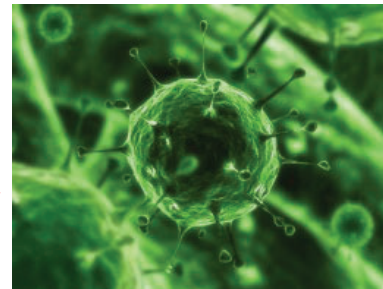
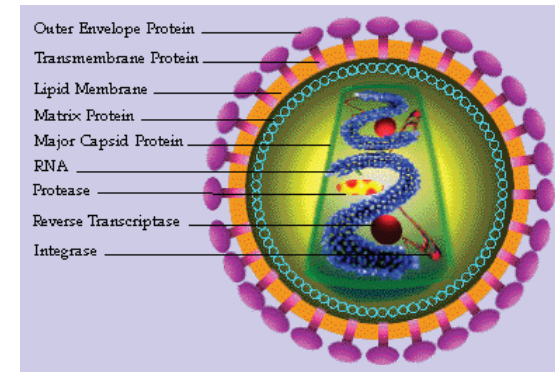
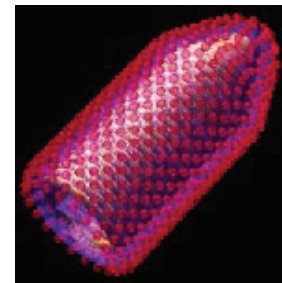
↳ animal plants fungi algae virus bacteria → viruses are host specific.  
 ↳ bacteria viruses called phage / bacteriophage.

↳ bacterio phage structure.

↳ nucleic acid (DNA) → never ever both  
 ↳ RNA → more dangerous.  
 ↳ protein coat capsid (shape vary)  
 ↳ enveloped → mostly animal viruses  
 ↳ kind bilayer.



- Acellular → difficult to handle  
 ↳ because when you target then you also target to host
- Consist of DNA or RNA core
- Core is surrounded by a protein coat → named capsid
- Coat may be enclosed in a lipid envelope generally animal viruses.
- Viruses replicate only when they are in a living host cell  
 ↳ host specific → animal viruses can only infect animals  
 ↳ can't infect plants



(e)

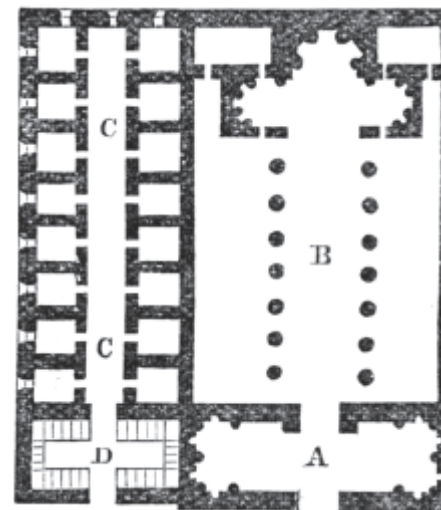
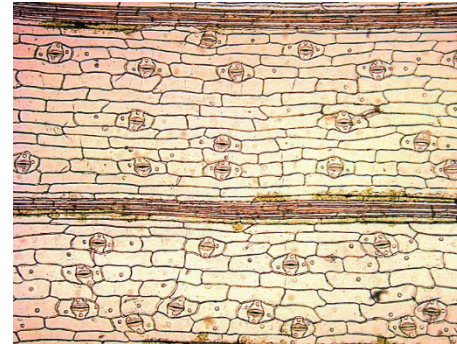
# Multicellular Animal Parasites

- Eukaryote
- Multicellular animals
- Parasitic flatworms and round worms are called **helminths**.
- Microscopic stages in life cycles.



# The First Observations

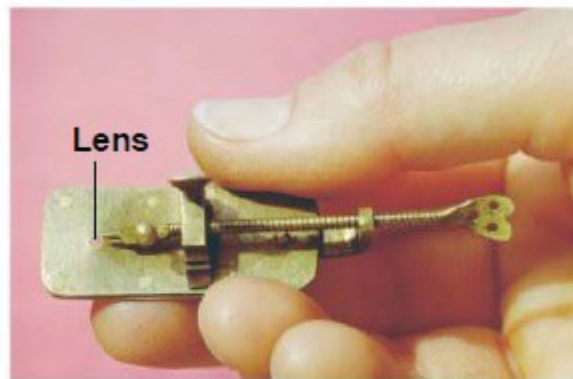
- In 1665, Robert Hooke reported that living things were composed of little boxes or cells.
- In 1858, Rudolf Virchow said cells arise from preexisting cells.
- Cell Theory. All living things are composed of cells and come from preexisting cells



# The First Observations

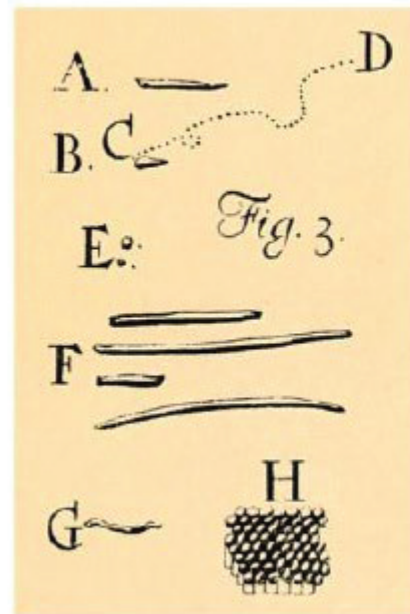
- 1673-1723, Antoni van Leeuwenhoek described live microorganisms that he observed in teeth scrapings, rain water, and peppercorn infusions.

The van Leeuwenhoek microscope

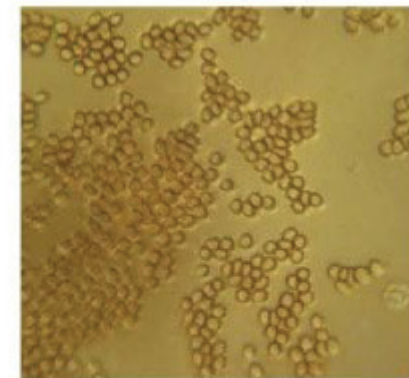


(a)

T. D. Brock



(b)



(c)

Brian J. Ford

# The Debate Over Spontaneous Generation

- The hypothesis that living organisms arise from nonliving matter is called spontaneous generation. According to spontaneous generation, a “vital force’ Forms life. *↳ abiogenesis*
- The Alternative hypothesis, that the living organisms arise from preexisting life, is called biogenesis.

# The Theory of Biogenesis

- ❑ 1861: Louis Pasteur demonstrated that microorganisms are present in the air.
- ❑ Pasteur's S-shaped flask kept microbes out but let air in.



# The Golden Age of Microbiology

- 1857-1914
- Beginning with Pasteur's work, discoveries included the relationship between microbes and disease, immunity, and antimicrobial drugs

# Fermentation and Pasteurization

- Pasteur showed that microbes are responsible for fermentation.
- Fermentation is the conversion of sugar to alcohol to make beer and wine.
- Microbial growth is also responsible for spoilage of food.
- Bacteria that use alcohol and produce acetic acid spoil wine by turning it to vinegar (acetic acid).

# Fermentation and Pasteurization

- Pasteur demonstrated that these spoilage bacteria could be killed by heat that was not hot enough to evaporate the alcohol in wine. This application of a high heat for a short time is called **pasteurization**.

↓ 62°C

↑

↓

→ in each step  
microorganisms minimized  
↳ to keep proteins we need lower degree than 100  
< 60°C



# The Germ Theory of Disease

- ❑ 1835: Agostino Bassi showed a silkworm disease was caused by a fungus.
- ❑ 1865: Pasteur believed that another silkworm disease was caused by a protozoan.
- ❑ 1840s: Ignaz Semmelwise advocated handwashing to prevent transmission of puerperal fever from one OB patient to another.

# The Germ Theory of Disease

- ❑ 1860s: Joseph Lister used a chemical disinfectant to prevent surgical wound infections after looking at Pasteur's work showing microbes are in the air, can spoil food, and cause animal diseases.
- ❑ 1876: Robert Koch provided proof that a bacterium causes anthrax and provided the experimental steps, Koch's postulates, used to prove that a specific microbe causes a specific disease.

*find the relationships  
between microbes and diseases.*

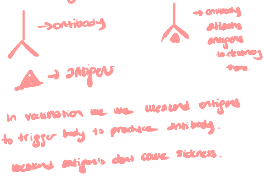
# The Germ Theory of Disease

## Koch's postulates

- Pathogen must be present in all cases of disease
- Pathogen must be isolated and grown in lab in pure culture
- Pathogen from pure cultures must cause disease when inoculated into healthy, susceptible lab animal
- Same pathogen must be isolated from the diseased lab animal

# Vaccination

immune system



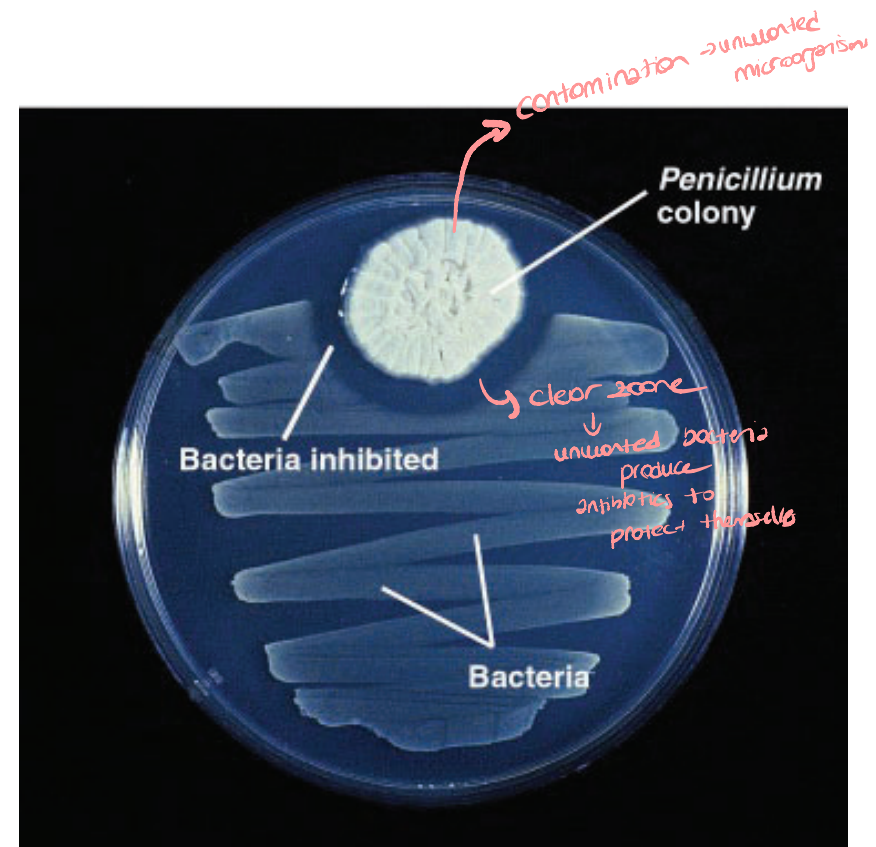
- 1796: Edward Jenner inoculated a person with cowpox virus. The person was then protected from smallpox.
- Called vaccination from *vacca* for cow
- The protection is called immunity

# The Birth of Modern Chemotherapy

- Treatment with chemicals is chemotherapy.
- Chemotherapeutic agents used to treat infectious disease can be synthetic drugs or antibiotics.
- Antibiotics are chemicals produced by bacteria and fungi that inhibit or kill other microbes.
- Quinine from tree bark was long used to treat malaria.
- 1910: Paul Ehrlich developed a synthetic arsenic drug, salvarsan, to treat syphilis.
- 1930s: Sulfonamides were synthesized.

# The Birth of Modern Chemotherapy

- 1928: Alexander Fleming discovered the first antibiotic.
- He observed that *Penicillium* fungus made an antibiotic, penicillin, that killed *S. aureus*.
- 1940s: Penicillin was tested clinically and mass produced.



# Modern Developments in Microbiology

In the 20th century, microbiology developed in two distinct directions:

- Applied and basic
- Molecular microbiology

Fueled by the genomics revolution

## Major Subdisciplines of Applied Microbiology

- Medical microbiology and immunology

Have roots in Koch's work

- Agricultural microbiology and industrial microbiology

Developed from concepts developed by Beijerinck and Winogradsky

- Aquatic microbiology and marine microbiology

Developed from advances in soil microbiology

- Microbial ecology

Emerged in 1960s–70s

# Modern Developments in Microbiology

## Basic Science Subdisciplines in Microbiology

### ➤ Microbial systematics

The science of grouping and classifying microorganisms

### ➤ Microbial physiology

Study of the nutrients that microbes require for metabolism and growth and the products that they generate

### ➤ Cytology

Study of cellular structure

### ➤ Microbial biochemistry

Study of microbial enzymes and chemical reactions

### ➤ Bacterial genetics

Study of heredity and variation in bacteria

### ➤ Virology

Study of viruses

# Modern Developments in Microbiology

## Basic Science Subdisciplines in Microbiology

- Molecular Microbiology
- Biotechnology

Manipulation of cellular genomes

DNA from one organism can be inserted into a bacterium and the proteins encoded by that DNA harvested

- **Genomics**: study of all of the genetic material (DNA) in living cells
- **Transcriptomics**: study of RNA patterns
- **Proteomics**: study of all the proteins produced by cell(s)
- **Metabolomics**: study of metabolic expression in cells
- **Recombinant DNA technology or genetic engineering** involves microbial genetics and molecular biology.

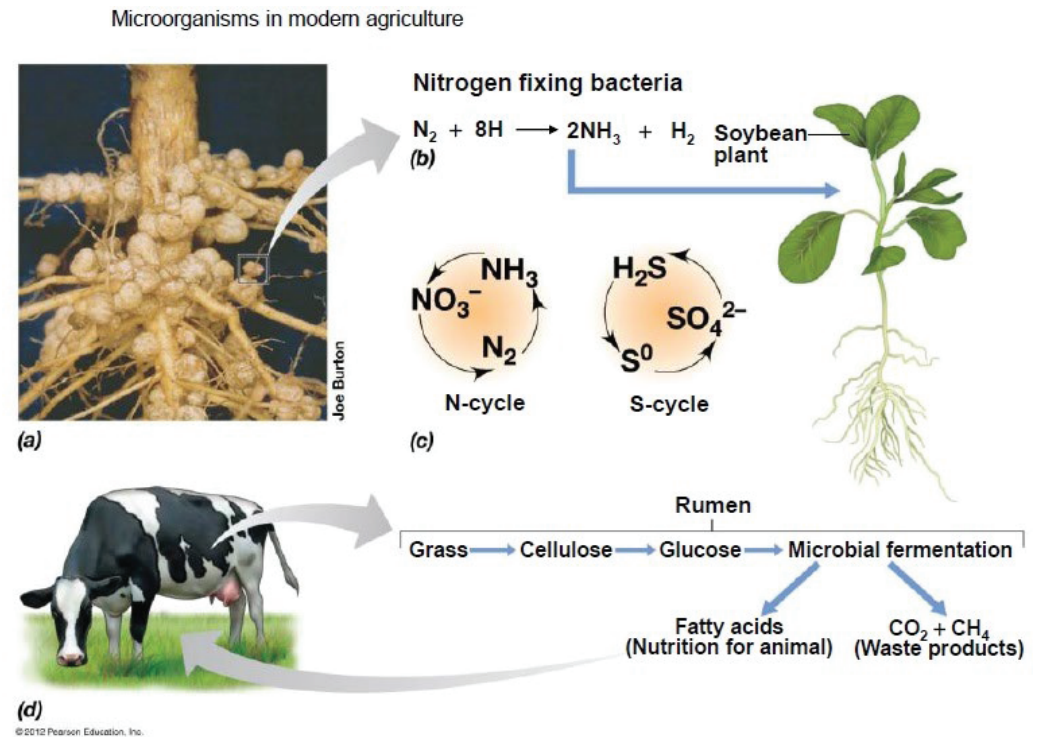
# Microbes and Human Welfare

- Microbial Ecology
- Bacteria recycle carbon, nutrients, sulfur, and phosphorus that can be used by plants and animals.

# Microorganisms and Agriculture

Many aspects of agriculture depend on microbial activities

- Positive impacts
  - nitrogen-fixing bacteria
  - cellulose-degrading microbes in the rumen
  - regeneration of nutrients in soil and water
- Negative impacts
  - diseases in plants and animals



# Microorganisms and Food

- Negative impacts
  - Food spoilage by microorganisms requires specialized preservation of many foods
- Positive impacts
  - Microbial transformations (typically fermentations) yield
    - dairy products (e.g., cheeses, yogurt, buttermilk)
    - other food products (e.g., sauerkraut, pickles, leavened breads, beer)

# Microorganisms, Energy, and the Environment

➤ The role of microbes in biofuels production

• For example, methane, ethanol, hydrogen

➤ The role of microbes in cleaning up pollutants (bioremediation)

some microorganisms phenol as a carbon source → toxic material } example.  
↓  
slow process } called  
bio-attenuation }  
↓  
enhance the speed of process.  
↑  
indigenous } add nutrients to system → make microbes adjust pH, temp, supply air/O<sub>2</sub> }  
↓  
these adjustments called "biostimulation"  
↑  
sometimes you can add microbes to the system from outside. its called bioaugmentation.

## Biofuels



John A. Breznak

(a)

Production of methane



(b)

# Bioremediation

- Bacteria degrade organic matter in sewage.
- Bacteria degrade or detoxify pollutants such as oil and mercury



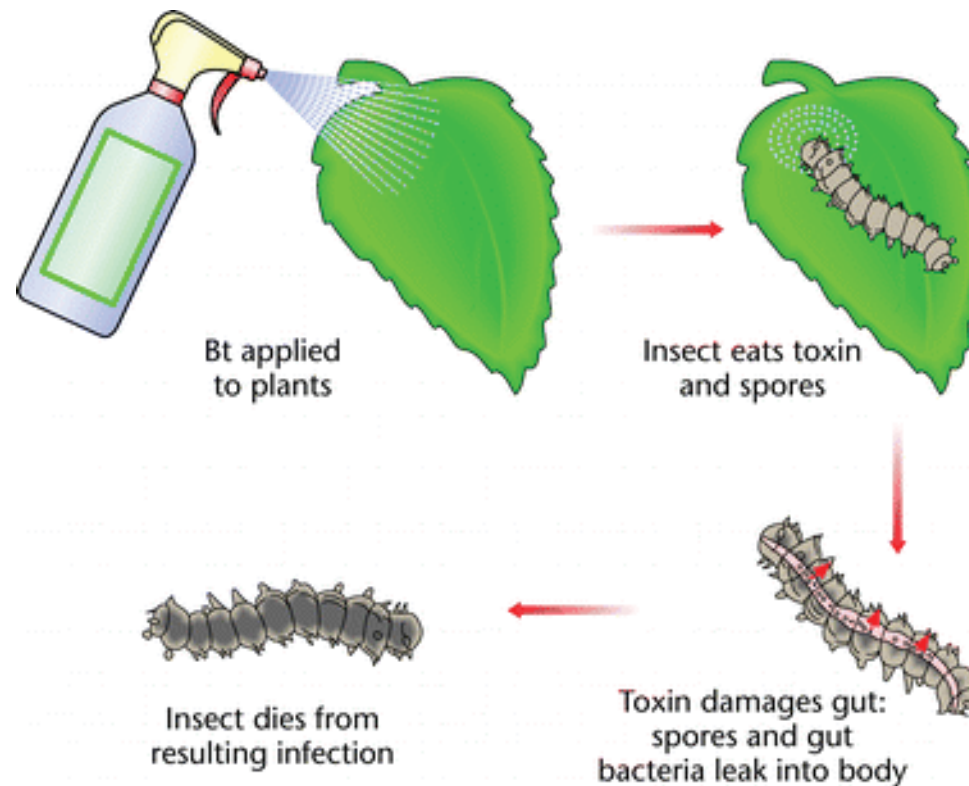
# Biological Insecticides

p persistent in the environment. → after they become pollution.

Xenobiotics → means man made chemicals.  
(pesticide, herbicide, weedicide)

- Microbes that are pathogenic to insects are alternatives to chemical pesticides to prevent insect damage to agricultural crops and disease transmission.
- *Bacillus thuringiensis* infections are fatal in many insects but harmless to other animals including humans and to plants.

B.t → its produce cry protein → active in gut of the insects → because gut is basic. (pH 8) → environmental friendly process. don't harm humans.



# Modern Biotechnology and Genetic Engineering

- Biotechnology, the use of microbes to produce foods and chemicals, is centuries old.
- Genetic engineering is a new technique for biotechnology. Through genetic engineering, bacteria and fungi can produce a variety of proteins including pharmaceuticals, vaccines and enzymes.
- Missing or defective genes in human cells can be replaced in gene therapy.
- Genetically modified bacteria are used to protect crops from insects and freezing.

# Normal Microbiota

- Normal microbiota prevent growth of pathogens.
- Normal microbiota produce growth factors such as folic acid and vitamin K.
- Resistance is the ability of the body to ward off disease.
- Resistance factors include skin, stomach acid, and antimicrobial chemicals.

# Infectious Diseases

- When a pathogen overcomes the host's resistance, disease results.
- Emerging Infectious Diseases (EID): New diseases and diseases increasing in incidence

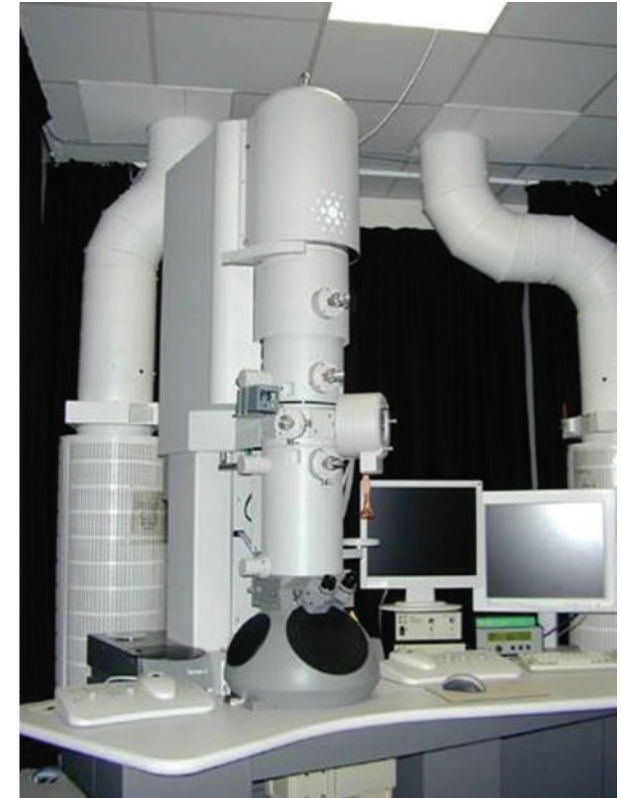
# Observing Microorganisms Through a Microscope

## Units of Measurement

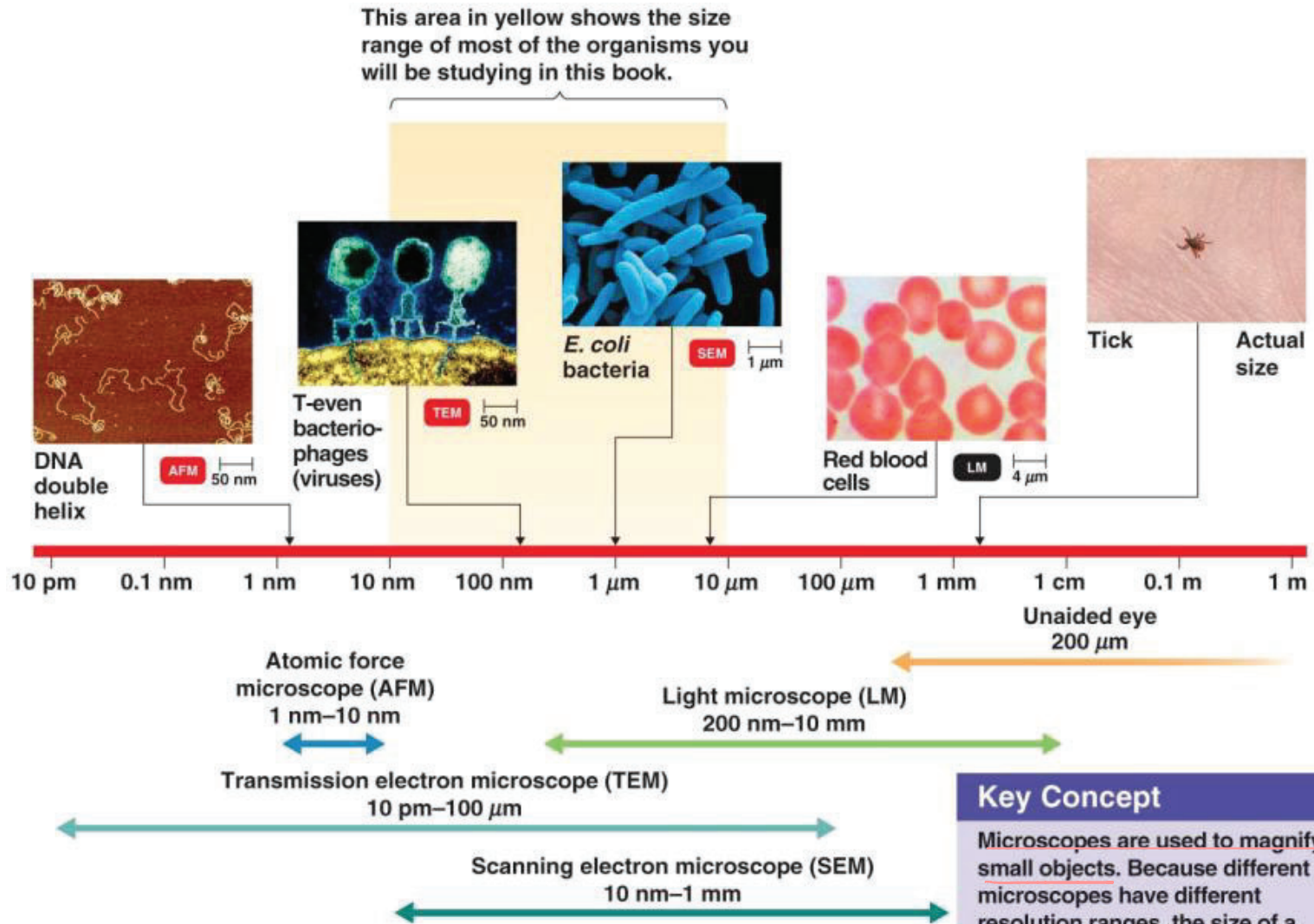
- $1 \mu\text{m} = 10^{-6} \text{ m} = 10^{-3} \text{ mm}$
- $1 \text{ nm} = 10^{-9} \text{ m} = 10^{-6} \mu\text{m}$
- $1000 \text{ nm} = 1 \mu\text{m}$
- $0.001 \mu\text{m} = 1 \text{ nm}$

Angstrom  $\rightarrow \text{Å} \ 10^{-10} \text{ m}$

most microbes  $0,2 \mu\text{m}$



# Observing Microorganisms Through a Microscope



**Key Concept**

Microscopes are used to magnify small objects. Because different microscopes have different resolution ranges, the size of a specimen determines which microscopes can be used to view the specimen effectively.

# Preparing Smears for Staining

- A thin film of a solution of microbes on a slide is a **smear**.
- A smear is usually fixed to attach the microbes to the slide and to kill the microbes.

↳ with air or heat  
↳ to keep on slide the smear



# Simple Stains

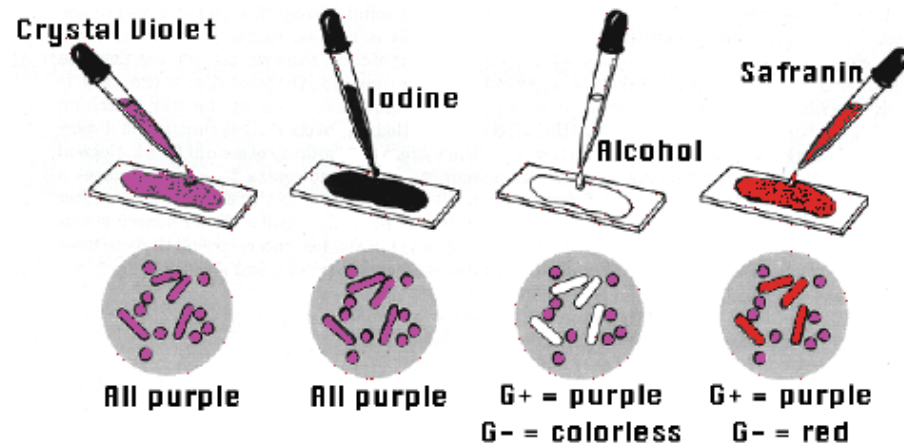
- Use of a single basic dye is called a simple stain.
- A mordant may be used to hold the stain or coat the specimen to enlarge it.

# Differential Stains: Gram Stain

- The Gram stain classifies bacteria into gram-positive and gram-negative.
- Gram-positive bacteria tend to be killed by penicillin and detergents.
- Gram-negative bacteria are more resistant to antibiotics.

# Gram Staining Procedure

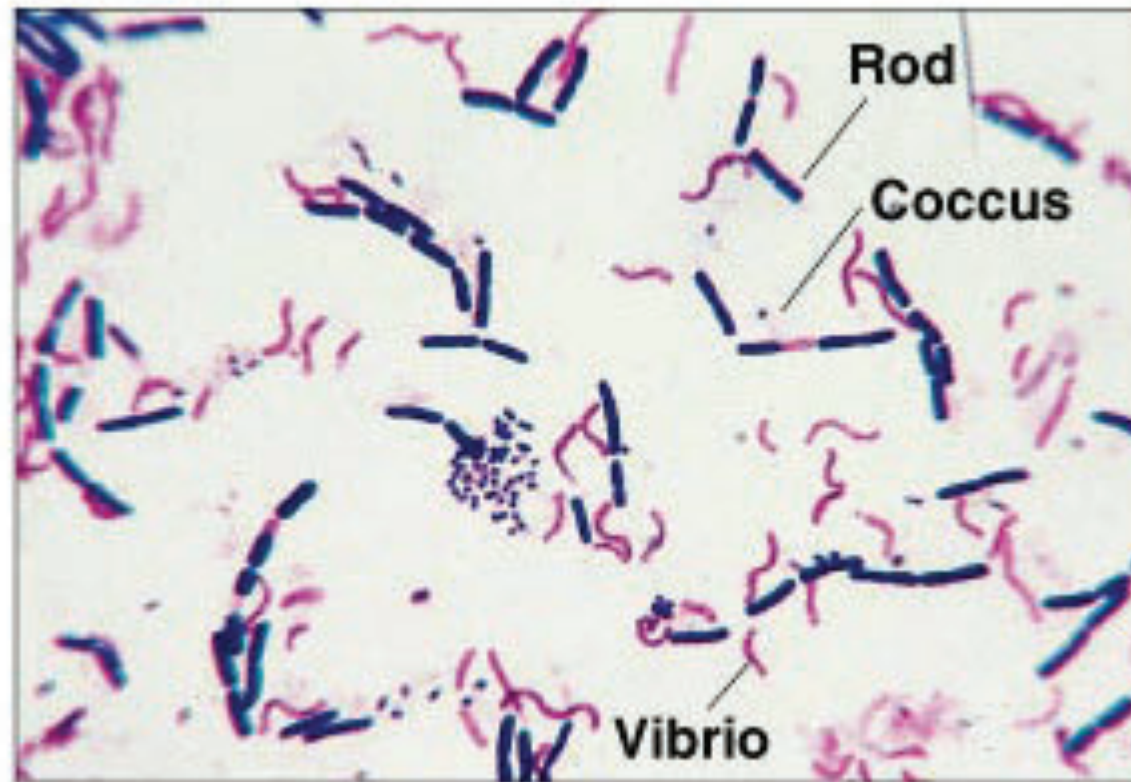
- A heat-fixed smear is covered with a basic purple dye (**usually crystal violet**)...the primary stain since it imparts its color to all cells
- The purple dye is washed off and the smear is covered with iodine (**the mordant**)...after rinsing both will appear purple still
- Slide is washed with alcohol or alcohol-acetone (**decolorizing agent**)...removes dye from Gram negative bacteria *since gram positive have more thick peptidoglycan layer → it will still have some color*
- Alcohol is rinsed, and the slide is counterstained with safranin (**a basic red dye**) then rinsed...Gram+ will be purple...Gram- will be red   
 *↓  
safranin*



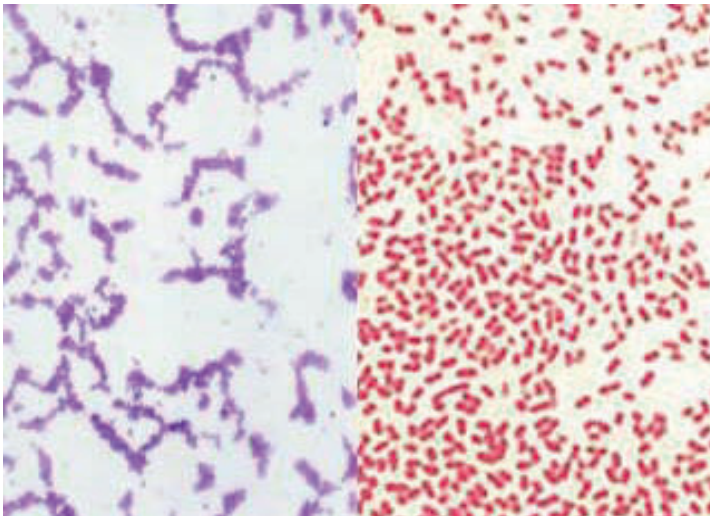
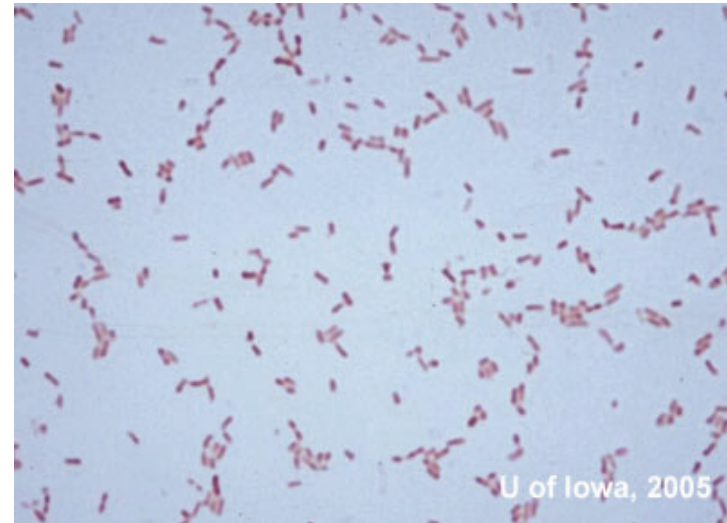
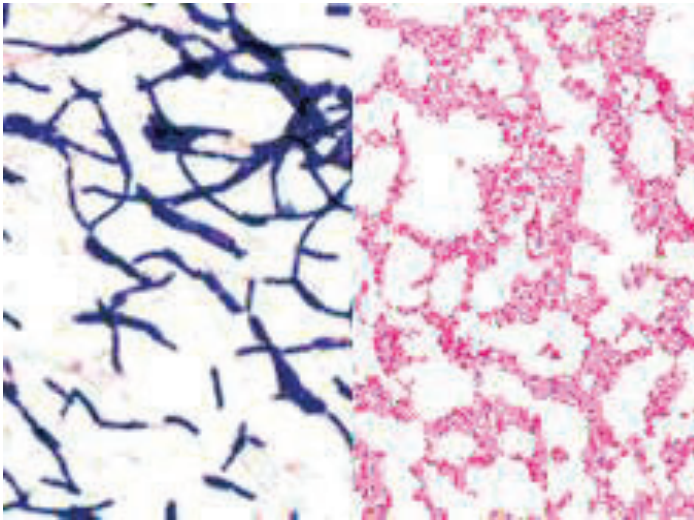
# Differential Stains: Gram Stain

	Color of Gram + cells	Color of Gram – cells
Primary stain: Crystal violet	Purple	Purple
Mordant: Iodine <i>↳ helps dye to stain the cell</i>	Purple	Purple
Decolorizing agent: Alcohol-acetone	Purple	Colorless
Counterstain: Safranin <i>↳ means secondary stain</i>	Purple	Red

# Differential Stains: Gram Stain



# Gram-Stained Bacteria



# Acid-Fast Stain

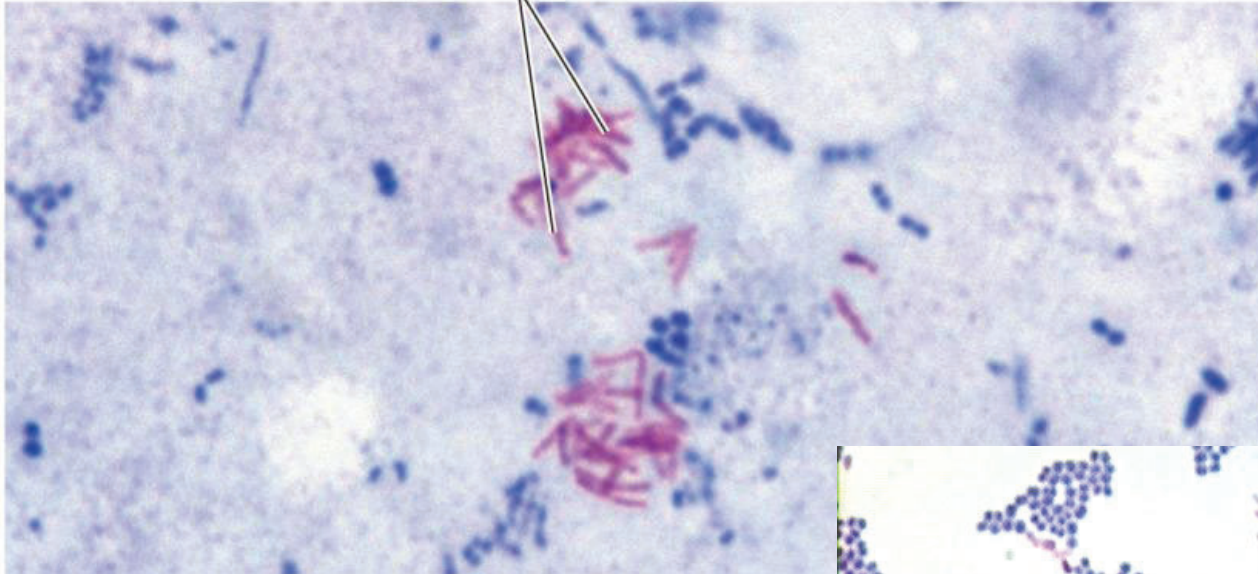
- Another differential staining technique
- Stain binds strongly to bacteria that have waxy material in their cell walls
  - stained waxy cell walls will not decolorized when rinsed with acid-alcohol
  - *Mycobacterium tuberculosis* & *Mycobacterium leprae*
  - *Nocardia sp.* – causes Nocardiosis a virulent form of pneumonia

# Acid-Fast Stain

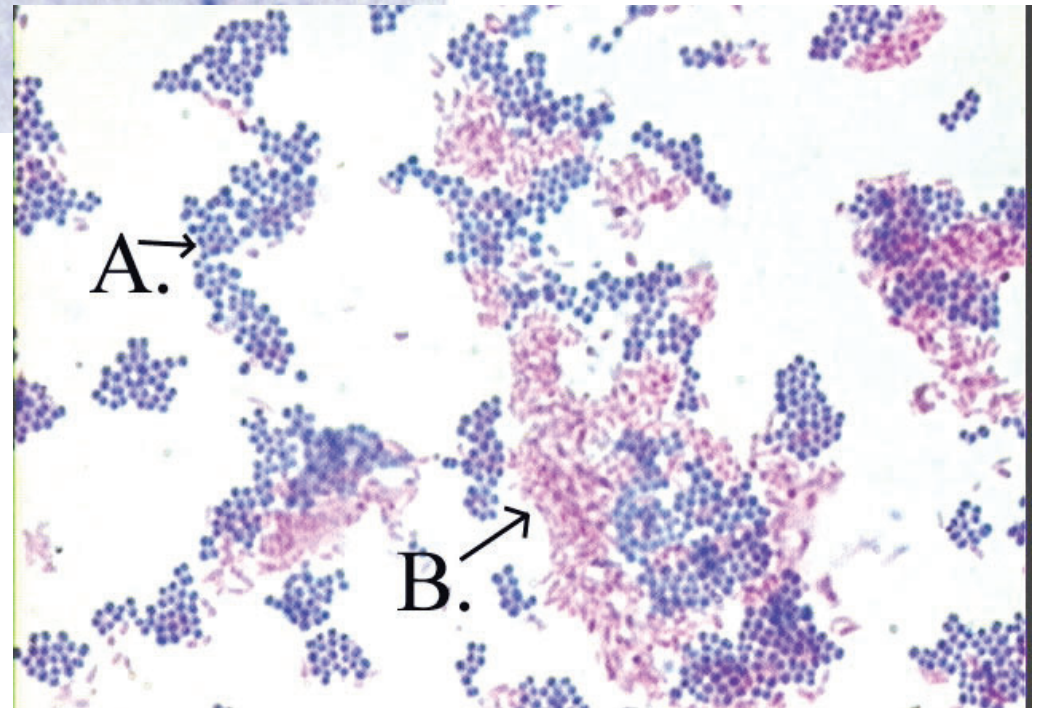
	<b>Color of Acid-fast</b>	<b>Color of Non-Acid-fast</b>
<b>Primary stain: Carbolfuchsin + Heat</b>	<b>Red</b>	<b>Red</b>
<b>Decolorizing agent: Acid-alcohol</b>	<b>Red</b>	<b>Colorless</b>
<b>Counterstain: Methylene blue</b>	<b>Red</b>	<b>Blue</b>

# Acid-Fast Bacteria

*M. leprae*



*A = non acid fast*  
*B = acid fast*



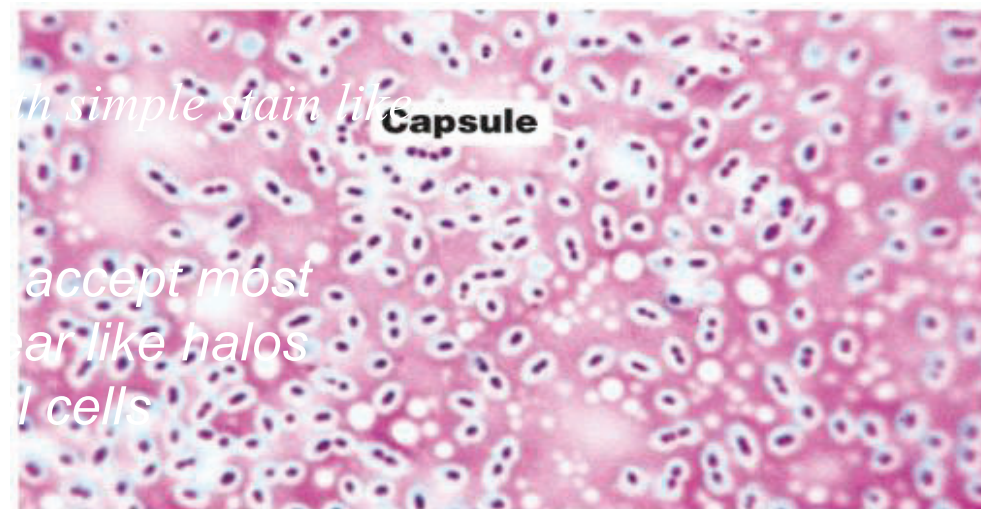
# Special Stains to Distinguish Parts of Cells

## Negative Staining for Capsules

- Many microorganisms have a gelatinous capsules that are difficult to dye
- Helps protect them from host phagocytosis often making them more virulent
- Stain slide with India Ink or Nigrosin for background contrast

if something antigenic it means it triggers immune system. So body produce antibodies.  
Ex: bacteria, viruses, protozoa

→ pathogenic  
↓  
love  
love



with simple stain like  
accept most  
as like halos  
of cells

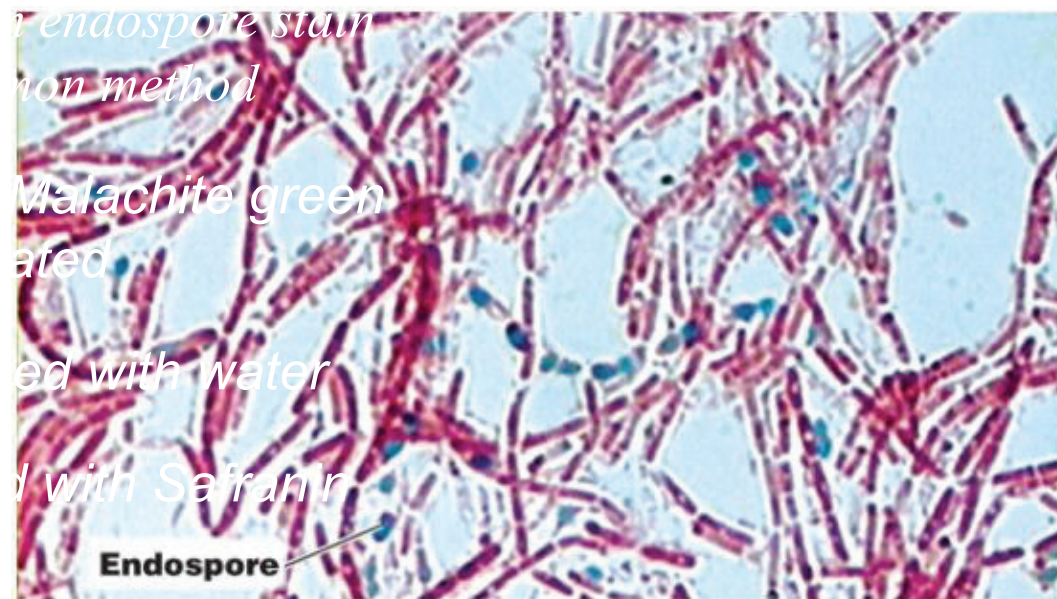
(a) Negative staining

LM 5 μm

# Special Stains to Distinguish Parts of Cells

## Endospore staining

- Endospores are dormant, resistant structures in some bacterial cells that protect them from adverse environmental conditions
- Cannot be stained with simple stains or Gram staining because dye will not penetrate endospore wall



**(b) Endospore staining**

LM 5µm

# Special Stains to Distinguish Parts of Cells

## Flagella stain

- Flagella are locomotive structures that are typically too small to see with a light microscope
- Using a mordant to enhance their diameter and Carbolfuchsin stain they can be observed



**(c)** Flagella staining

LM  5  $\mu$ m