BIOL 251: BASIC MICROBIOLOGY 2020/2021

Name: Dr. (Mrs.) Linda Aurelia Ofori
Email: laandoh.cos@knust.edu.gh /aurelia012001@yahoo.com
Phone No: 0244220064
Room number: **016**
Department: Theoretical and Applied Biology
Faculty & College: Biosciences, Science

KNUST COVID-19 AWARENESS

COVID-19: Caused by a virus known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Spreads very **easily** from person to person.

Signs and symptoms: Fever or chills, cough, difficulty in breathing, cold, headache, diarrhoea, loss of taste/smell, and several non-specific symptoms.

Transmission: Respiratory droplets, airborne, contaminated surfaces.

Prevention: Adhere to the KNUST COVID-19 safety protocols

- Respiratory hygiene: Wear a nose mask, cough etiquettes
- Hand hygiene: Frequent hand washing, hand sanitizing
- Maintain 'safe' physical distancing
- Avoid crowds and confined/poorly ventilated spaces

Virus is changing itself with even more serious ramifications, so it is important we all adhere to the safety protocols

Course outline

- Introduction to the Science of Microbiology
- Nature and kinds of microorganisms
- Nutrition and growth of microorganisms
- Isolation, characterization and staining
- Culture of microorganisms
- Bacteria characterization

Learning Objectives – BIOL 251

1. Explain the different categories of microorganisms
2. Discuss the contributions microbes make to life
3. Discuss the origins of Microbiology
4. Describe the nature and kinds of microorganisms
5. Discuss the physiology of bacteria cell (nutrition, growth and metabolism)
6. Explain what modern medicine is all about
7. Acquire and show competency in routine lab skills applicable to microbiological methods (accurately reporting observations and analysis)

Course activities

- Lectures/discussions
- Audio-visual presentations
- Demonstrations
- Web-based instructional media and other educationally sound practices
- Comprehensive laboratory exercises (A laboratory manual will be provided).

Assessment Requirements

- ✓ **Punctuality, Attendance**
- ✓ **Presentations (Group)**
- ✓ **Assignments (Individual and Group)**
- ✓ **Short Quizzes – Un/announced**
- ✓ **Mid Semester Examination**
- ✓ **Practical Assignments – Weekly**

Continuous Assessment 30%

- ✓ **End of Semester Examination 70%**

Some Class expectations

- **To attend all lectures and practicals and on time**
- **Once lecture begins you will not be allowed to enter the lecture room**
- **To answer questions pertaining to the course of study during lectures**
- **To revise previous lecture notes prior to the next lecture**

❖ **Do NOT use your cell phone in class. It should be *placed on* silent or switched off as you enter the lecture room**

Recommended texts for further reading

- Introduction to Microbiology – Ingraham and Ingraham
- MICROBIOLOGY - Prescott, Harley and Klein
- Fundamentals of Microbiology – Frobisher
- Any good Microbiology book
- Authentic Microbiology journals/Papers

Requirements for practical classes

- ✓ A **Laboratory (practical) file** is essential. Any hard cover file with A4 paper to fit will be appropriate. In it you should record all protocols, diagrams, observations and results of practical assignments.

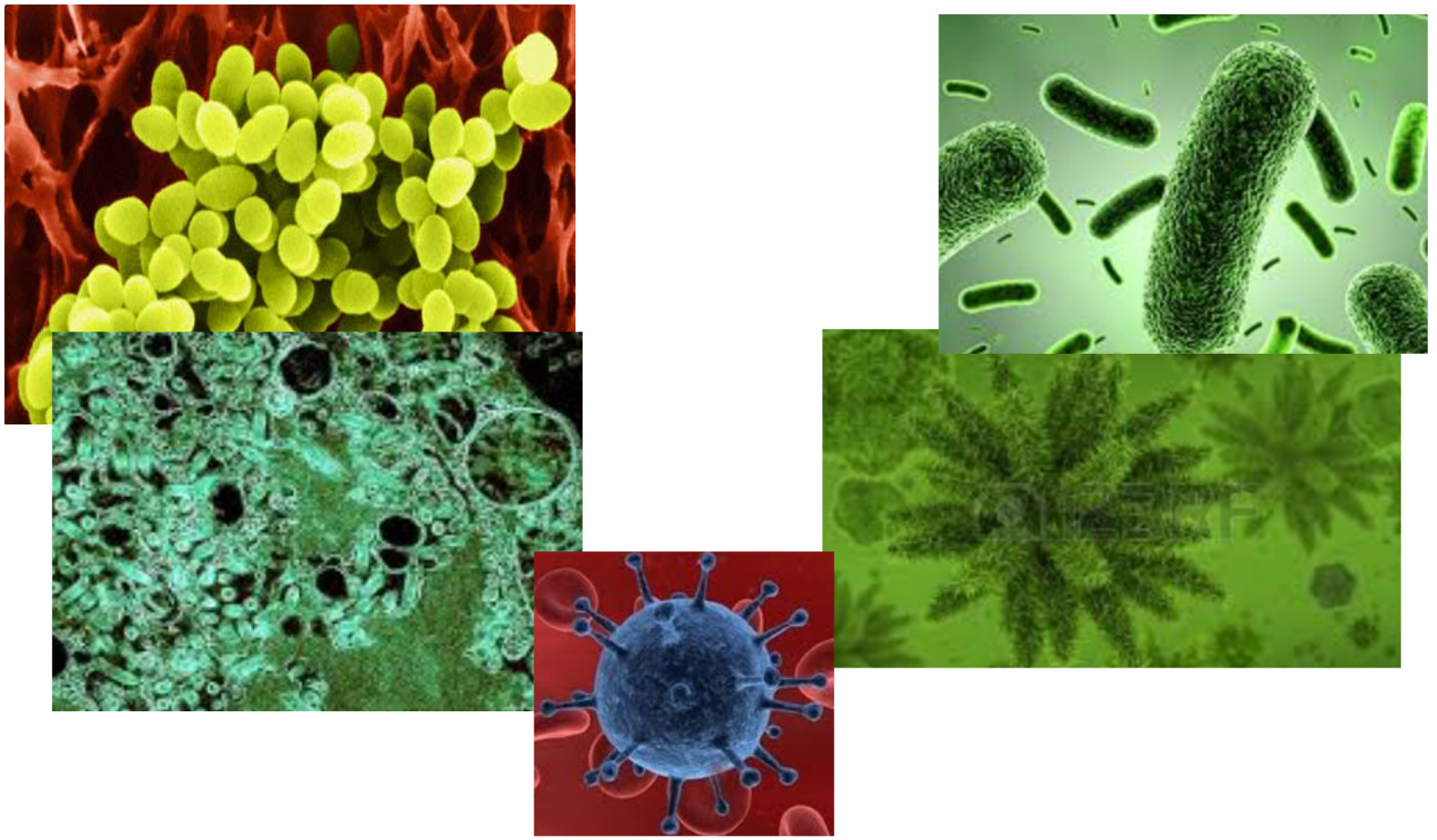
- ✓ Diagrams must be drawn with a **pencil**. Use pencils with soft lead, preferably HB. Label and rule lines in pencil, as this will facilitate correction.

Introduction to the Science of Microbiology

What is Microbiology?

Study of microbes/microorganisms

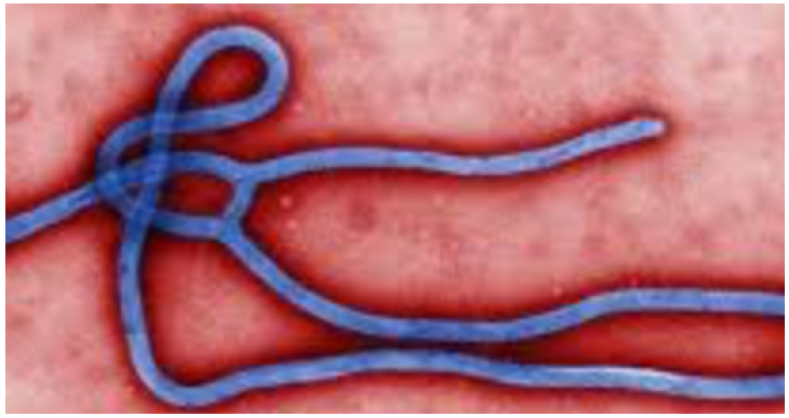
Microorganisms are minute living creatures that individually are too small to be seen with unaided/naked eye.

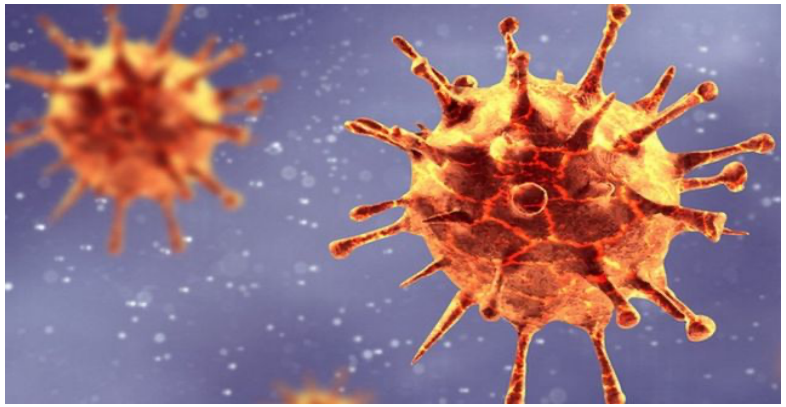


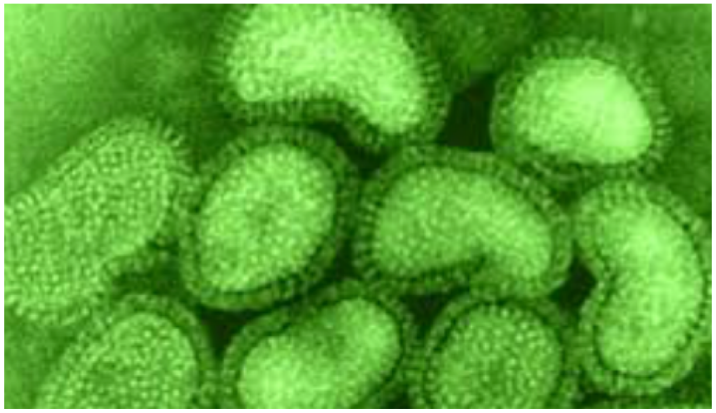
Microorganisms include:

- Viral
- Bacteria
- Fungi
- Protozoa
- Cyanobacteria (Blue-green algae)

Viral

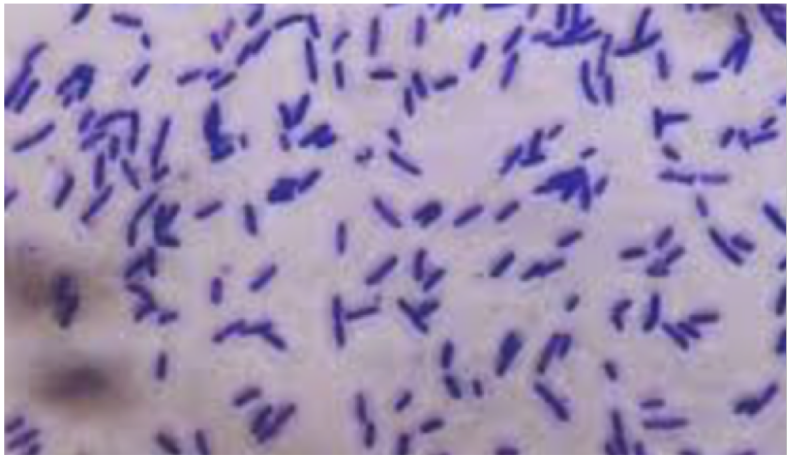






Hepatitis A, B,C,D,E virus, Influenza virus, Coronavirus, Ebola , Rabies virus

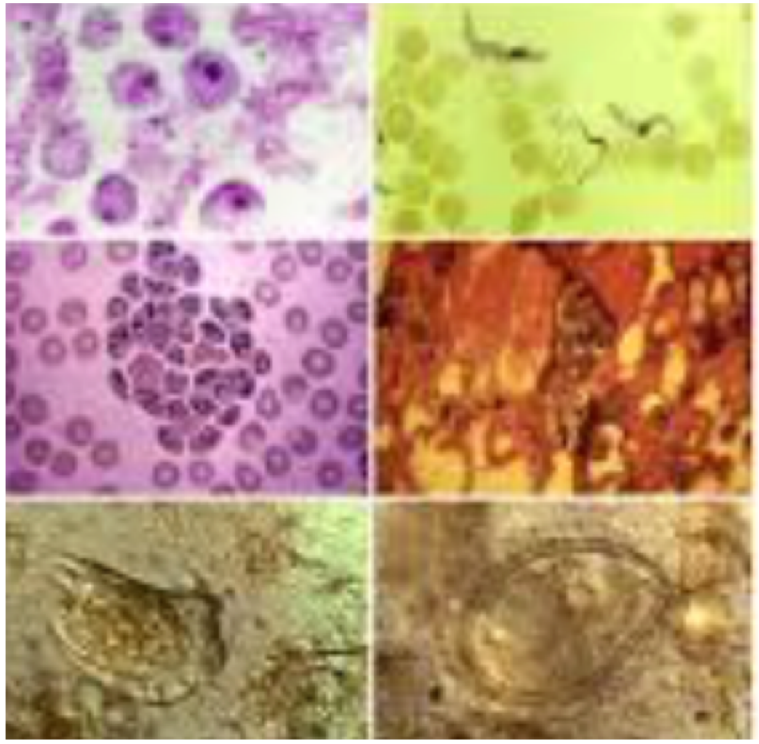
Bacteria

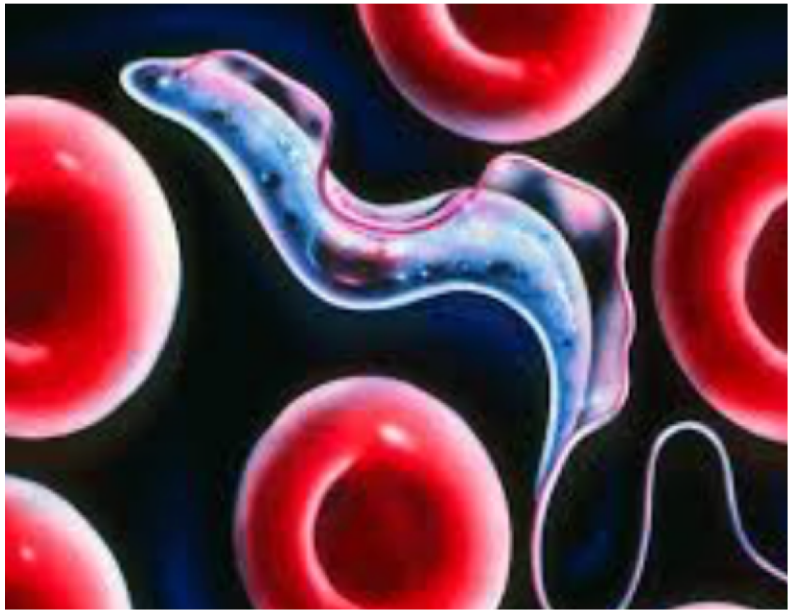


Staphylococcus, Streptococcus, Mycobacterium

Fungi

Protozoa





Cyanobacteria

Diseases they cause

VIRAL

- Poliomyelitis, COVID-19, Hepatitis, Influenza, foot and mouth disease, Rhinderpest, Rabies, African horse sickness

BACTERIA

- Brucella, Diphtheria, Cholera, Typhoid, Tuberculosis, Leprosy, Legionellosis

FUNGI

- Athlete's foot, Ringworm, Yeast infection (*Malassezia pachydermatis)*

PROTOZOA

- Malaria, Giardiasis, trypanosomiasis, leishmaniasis

CYANOBACTERIA (Blue-green algae)

- Swimmers allergies, nephritis.

Where can we find them?

- Microorganisms are found **everywhere** in our environment

- They are part of the human environment
  - Air
  - Soil
  - Water

- Majority of microbes do not cause disease
- Instead they are used to promote our well-being
- This explains the various branches of microbiology.

Medical Microbiology

deals mainly with diseases

- Pathogens are disease causing microbes
- Microbes can either produce more of itself to cause the problem or produce chemicals or products (usually called a **Toxin**) to interfere with the normal processes
- Non disease causing microorganisms are called **COMMENSALS**
- Most microorganisms are **OPPORTUNISTS**

Food Microbiology

- Food: Microbes as food: mushrooms, algae, single cell protein

- Microbes in food production: cheese, yoghurt, bread, sauerkraut, beer, wine, vinegar, pickle, soya sauce.

- Food processing: canning, pasteurization, freezing, irradiation, packaging, transport, ripening, spoilage (decay and disease).

Agricultural Microbiology

- Viral, bacterial and fungal diseases of animals and crop plants

- Beneficial symbiosis: bacteria and protozoa in ruminants, bacterial nitrogen fixation in legumes, fungal (mycorrhizal) associations with plants, cyanobacteria in *Azolla*.

- Composting, ensilaging, biological control of weeds by fungi and insects by bacteria and genetic engineering of plants with *Agrobacterium*.

Applied Microbiology

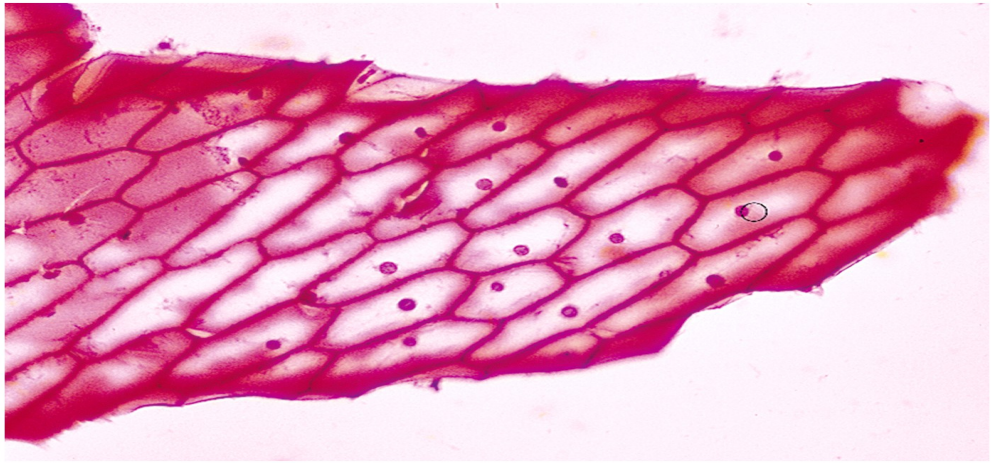
- Sewage disposal, oil spill clean ups (bioremediation)
- Methanogenesis (rubbish tip explosions)
- pesticide breakdown
- microbial fertilizers, water supply industry, single cell protein , fine chemicals, antibiotics, vitamins, alcohol, flavorings, enzymes, quality control, basis of biotechnology
- Recycling of nutrients e.g. carbon, nitrogen (nitrogen fixation, proteolysis, ammonification, nitrification, denitrification), sulfur, phosphorus, primary production in oceans and lakes, grazing

History of Microbiology – How it all started

Discovery of Cells

Robert Hooke (mid-1600s) - **1660s**

- Observed sliver of cork
- Saw “row of empty boxes”
- Coined the term cell



Description of microbes

1670s - Antonie van Leeuwenhoek,
'The father of Microbiology'

First real Microbiology Studies

van Leeuwenhoek was a true scientist and chronicled his observations:

- Microbes in wine and beer
- Impact of pepper on microbes
- Asked Hooke to confirm findings (review)
- Microbes in rainwater

Louis Pasteur (1822-1895)

In the 1800s the French were wondering why their wines were turning sour. Fermentation????

Prevailing theory: **fermentation was purely a chemical process with no input by living organisms.**

Pasteur used **experimentation** to provide evidence against this...

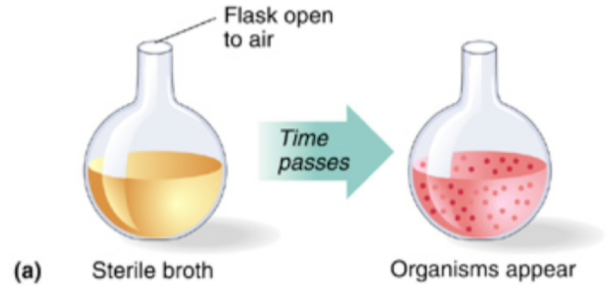
- Pasteur showed that **tiny organisms** were found in the wine.
- Sour wines contained populations of the microorganisms described by van Leeuwenhoek.
- When the organisms were killed through heating, **no fermentation would take place**.
- When the organisms were added back, **fermentation would again occur**.

Are microbes in the air responsible for spoilage?

- Louis Pasteur's grape juice went sour on standing for sometime
- Heated & Bottled, tightly closed, it remained the same
- However left ajar went sour
- Certain microbes in the air responsible for the spoilage.
- Breakthroughs in microbiology was the development of the Koch's postulates (1881).

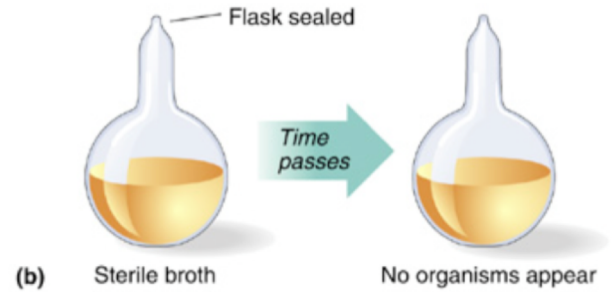
Louis Pasteur's Experiment

Each experiment begins with sterilized broth. Any living things the broth may have contained have been destroyed by heat.



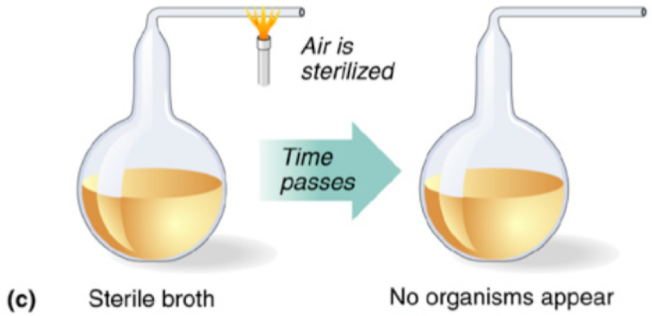
Pasteur: The broth provides a nutrient medium for the growth of unseen organisms in the air: life comes from other life.

His critics: A sterilized broth gives rise to life: spontaneous generation.



Pasteur: The heat has killed the microorganisms in the air.

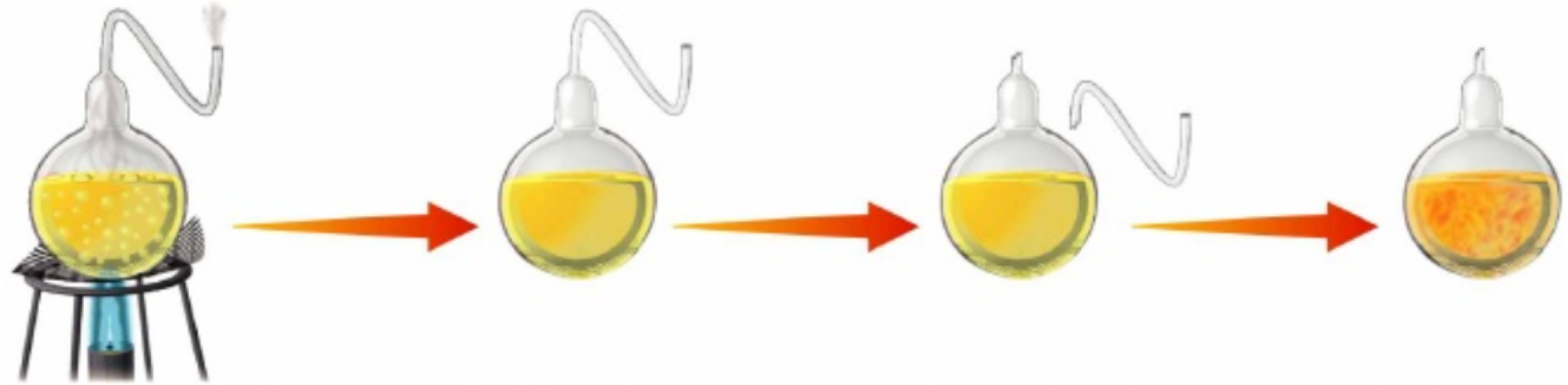
His critics: Sealing the flask prevents entry of the "life force."



Pasteur: The heat has killed the microorganisms in the air.

His critics: Sterilizing the air kills the "life force."

Louis Pasteur's modification – swan-necked flasks



Real question……Could microorganisms play a role in human health?

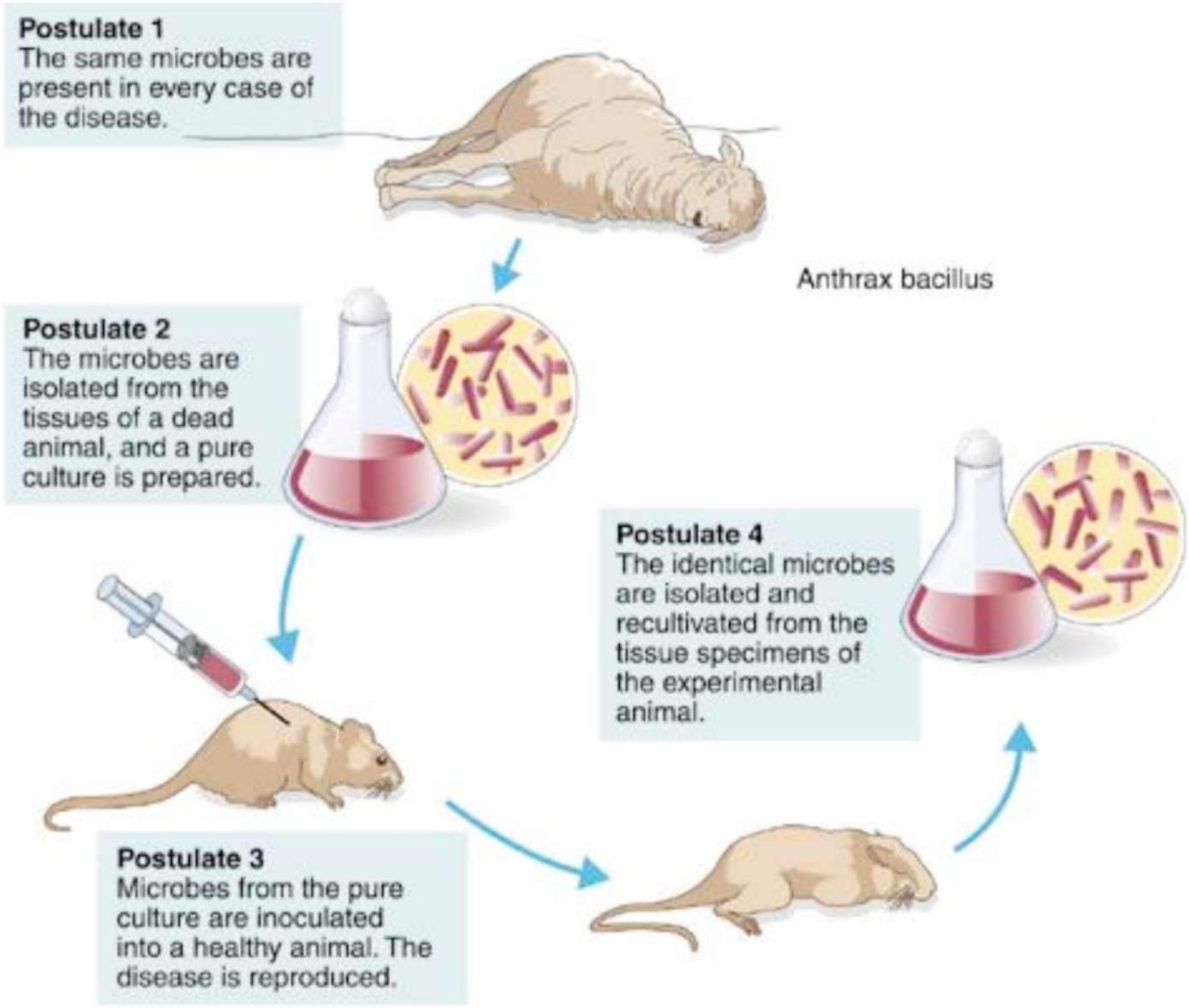
- Reasoning: Microbes could be transmitted to humans. This led him to propose the **germ theory of disease,** which states that ***microorganisms play significant roles in the development of infectious disease***.

- Diseased tissues often yield more than one microbes
- Consequently, not always obvious which microbe is the cause of disease
- Big problem in medical science in the 19th century
- Robert Koch Rules-of-proof of causality

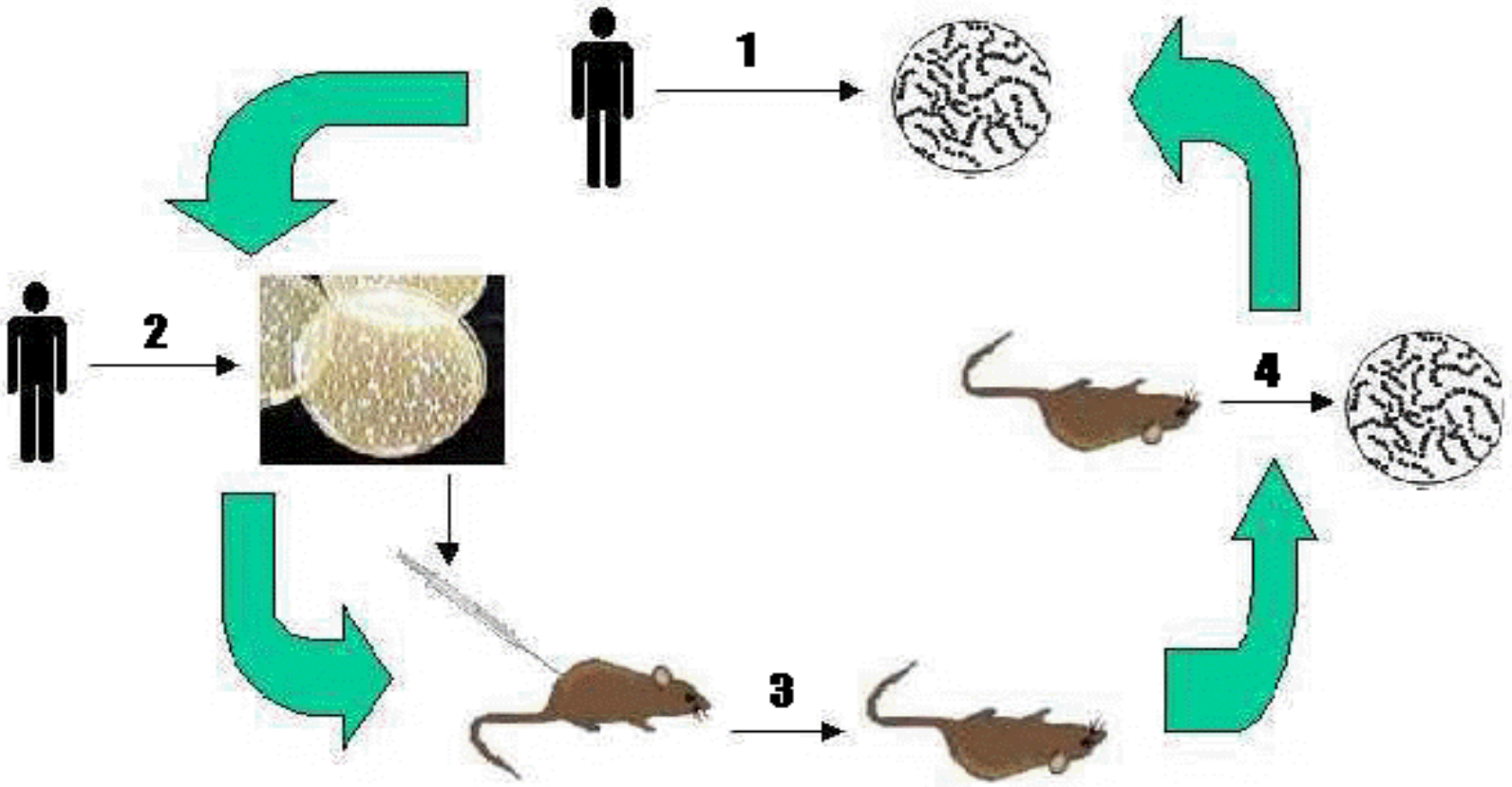
Nature of Infectious Disease
Robert Koch – 1843-1910

Koch's Postulates

- The organism in question must always be found associated with a particular disease
- The organism must be isolated and grown in pure culture.
- The organism grown in pure culture must be inoculated into a healthy host under favourable conditions and induce a characteristic disease.
- The organism must be re-isolated from the second host and compared with the first culture
- Both the diseased condition produced by inoculation and the organisms recovered from the inoculated host must correspond to the original diseased condition and to the first organisms isolated, respectively.


Postulate 1
The same microbes are present in every case of the disease.
Anthrax bacillus
Postulate 2
The microbes are isolated from the tissues of a dead animal, and a pure culture is prepared.
Postulate 4
The identical microbes are isolated and recultivated from the tissue specimens of the experimental animal.
Postulate 3
Microbes from the pure culture are inoculated into a healthy animal. The disease is reproduced.

A schematic diagram of Koch's postulate



NATURE AND KINDS OF MICROBES

- Prokaryotic cells (bacteria)
- Eukaryotic cells (plant and animal)

Prokaryotes differ from eukaryotes cells in several ways

Differences between prokaryotic and eukaryotic cells

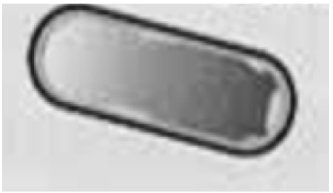
FEATURE	PROKARYOTES	EUKARYOTES
Cell size	small > 2 μm	larger than 2 μm
Nuclear membrane	-	+
# chromosomes	1	more than 1
Mitosis	-	+
Organelles	-	chloroplast & mitochondria
Endoplasmic reticulum	-	+
Cell wall	thin & usually peptidoglycan	thick or absent chemically diverse
Cytoplasmic Ribosomes	70s	80s
Organella Ribosomes	-	70s
Cilia	-	+
Flagella	+	+
	3-threaded helices	9:2 fibril arrangement

PROKARYOTIC DIVERSITY

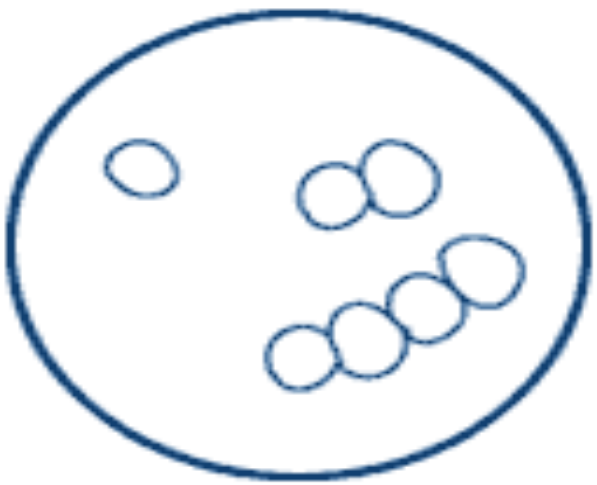
- Bacteria are differentiated mainly by their different **morphological**, **physiological** and **behavioral** characteristics.
  - Morphology (Shape)
  - Chemical composition (staining reactions)
  - Nutritional requirements
  - Biochemical activities and some source of energy (sunlight and chemicals)

Prokaryotic Diversity – Morphological Diversity

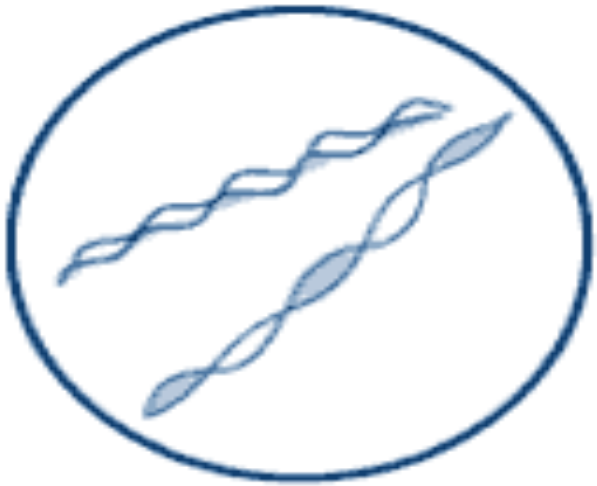
Three major forms of bacteria



- **Spherical or round forms**
  - Coccus (Plural- Cocci)
- **Rod-shaped forms**
  - Bacillus (Plural-Bacilli)
- **Spiral forms** or Spirillum/twisted - like corkscrew



Cocci

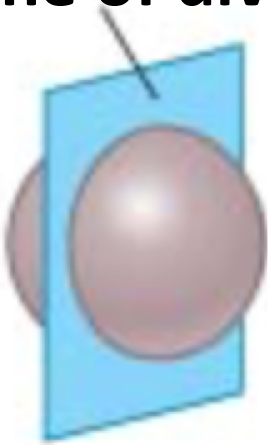


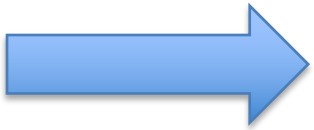
Spirilla

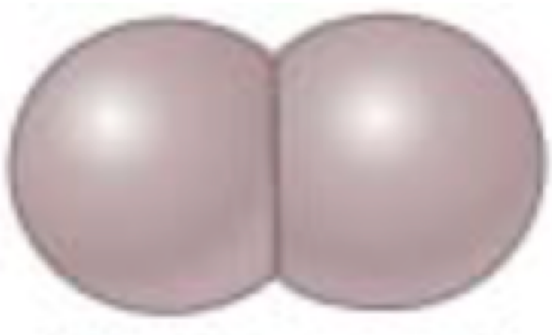
Bacilli

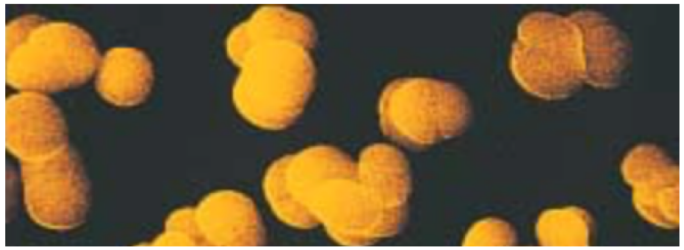
Arrangements after cell division (Cocci)

Plane of division







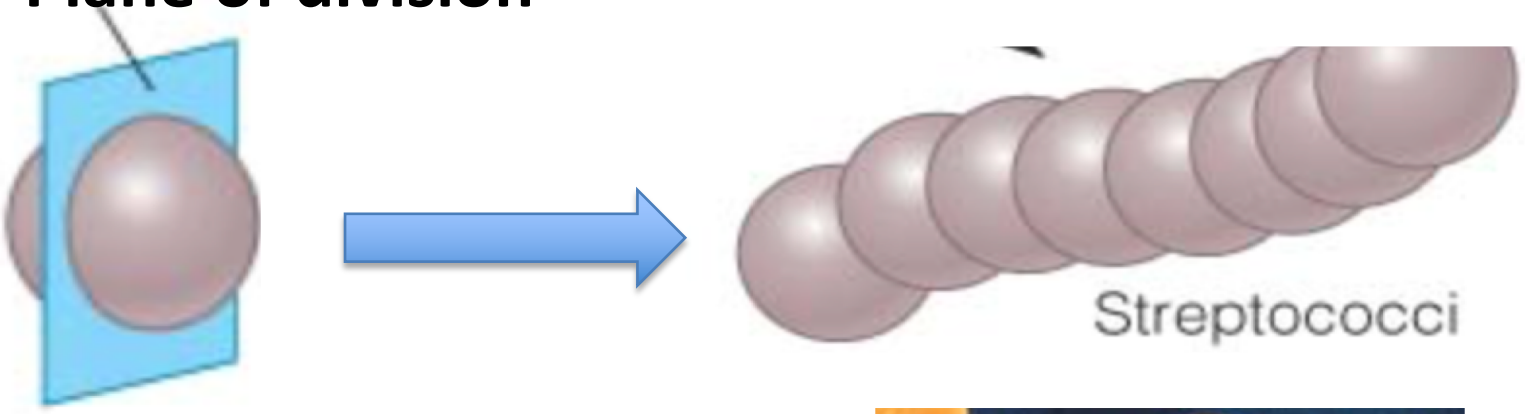


Cocci that divide and **remain in pairs** after dividing are

Diplococcus/Diplococci

Neisseria gonorrhoeae

Plane of division

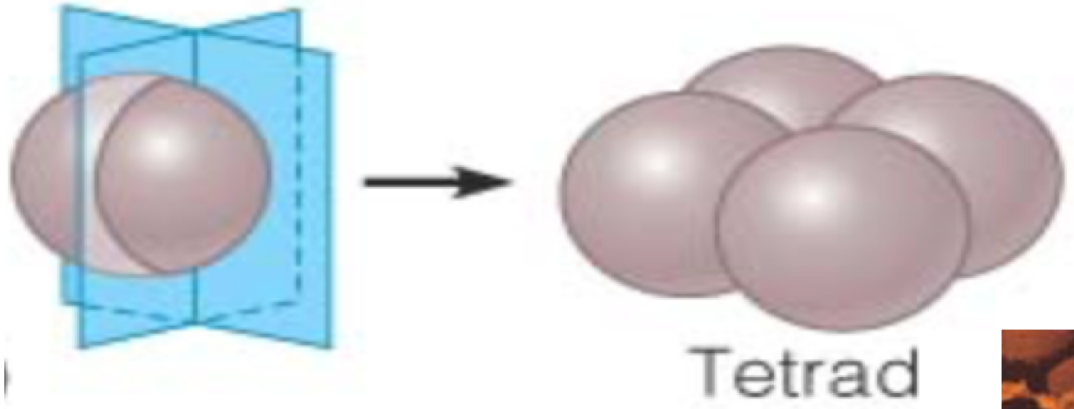


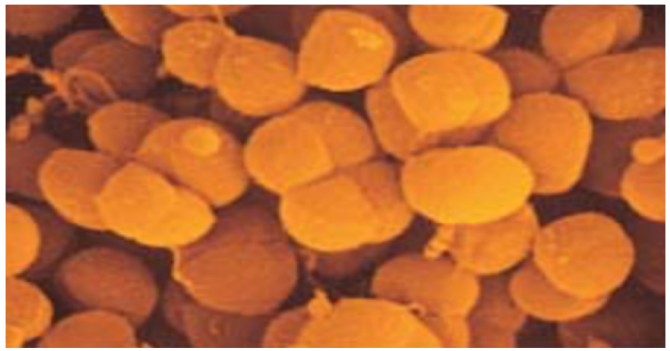
Divide and **remain attached in chainlike** patterns are

Streptococcus/Streptococci

Streptococcus pyogenes

Plane of division

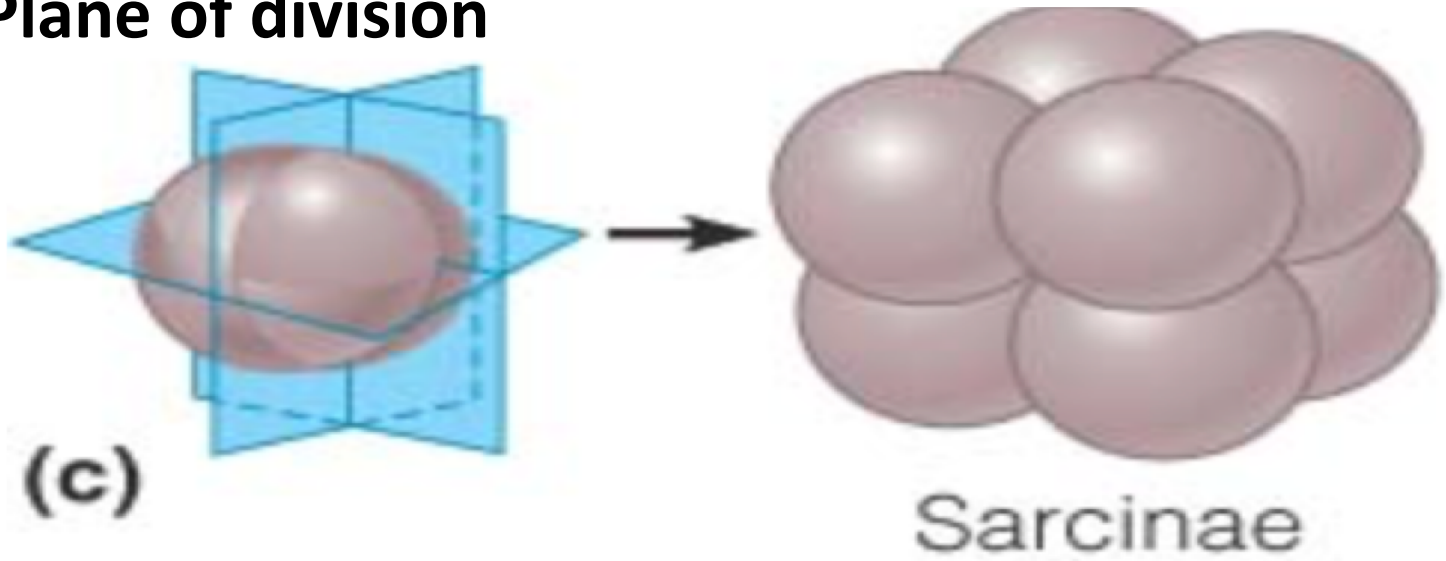


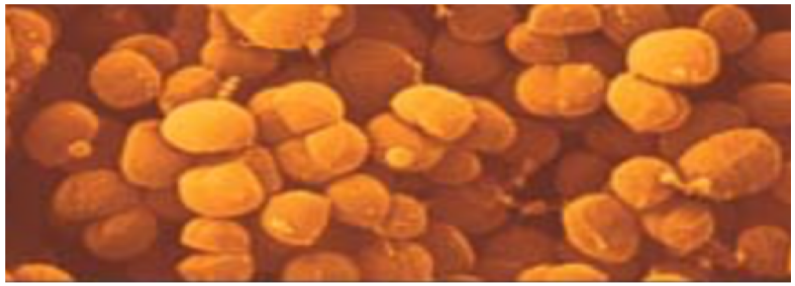


Those that divide in **two planes** and remain in **groups of four** are **Tetrads**

Micrococcus species

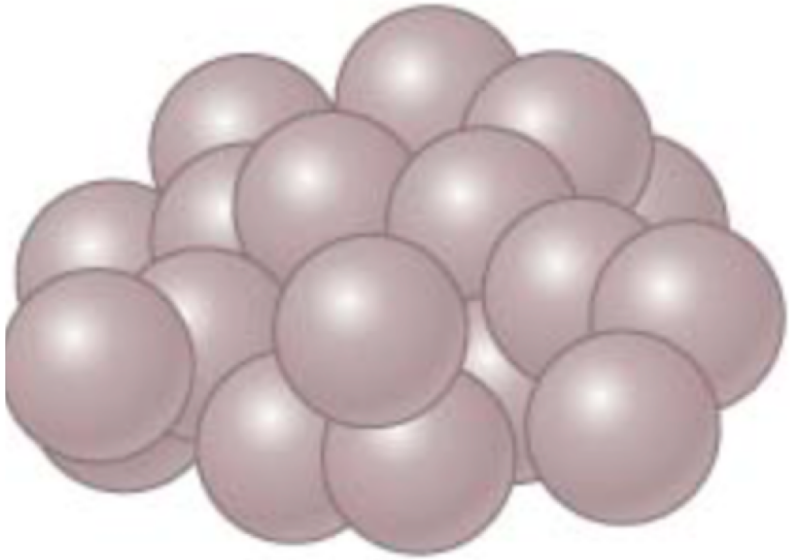
Plane of division





Cocci that divide in **three planes** and remain attached in **cube-like groups of eight** are called **Sarcina**

Sarcina ventriculi

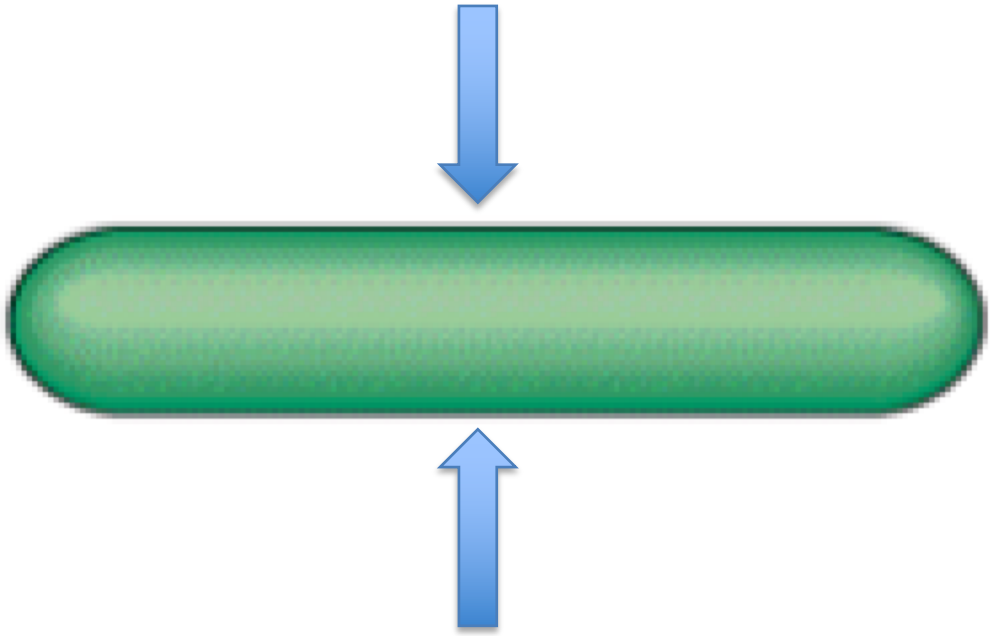


Cocci that divide in multiple planes and form grape like clusters or sheets are called Staphylococci

Staphylococcus aureus

Arrangements after cell division (Bacilli)

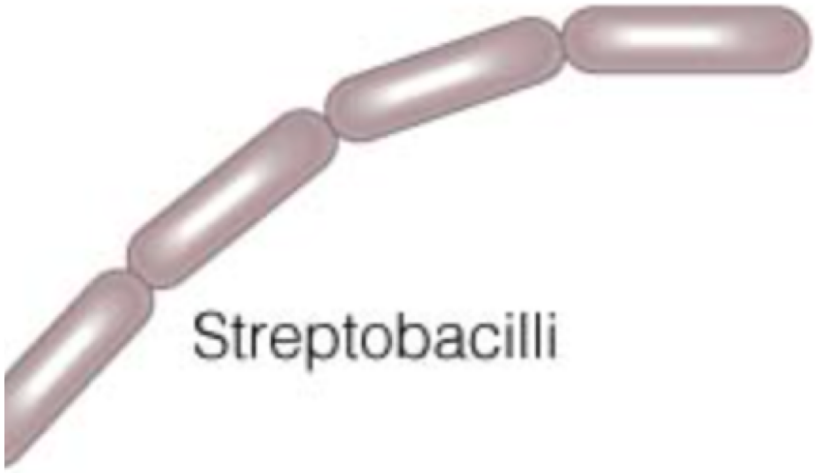
Bacilli can only divide **across their short axis** and there can be very few groupings



Single bacillus

Diplobacilli

Most bacilli appear as single rods. Diplobacilli appear in **pairs** after division.

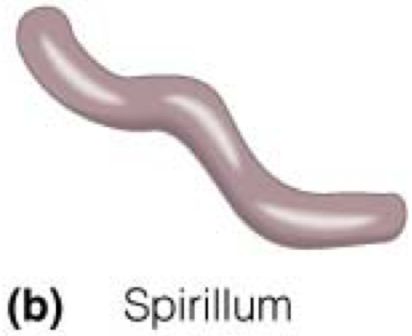


Streptobacilli appear in **chains** after division

Spiral bacteria forms

Helical and flexible

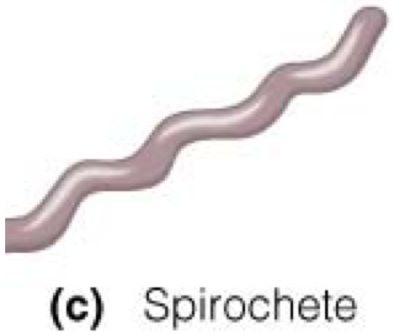
Spirillum

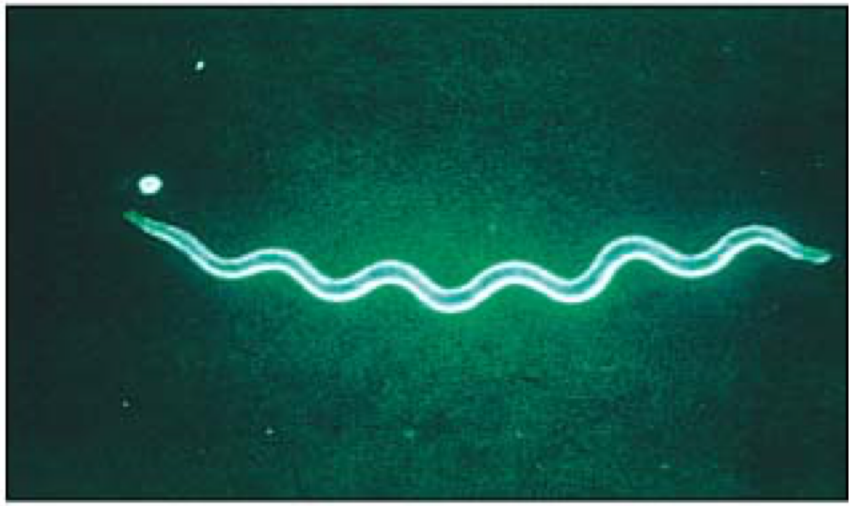


Spirilla have a helical shape and fairly rigid bodies and use whip-like external appendages called **flagella** to move

Vibrios: curved at one end like a comma

They look like **curved rods**

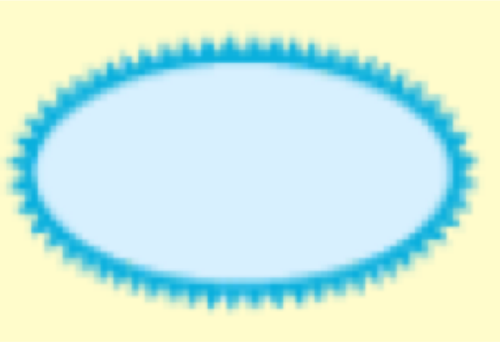


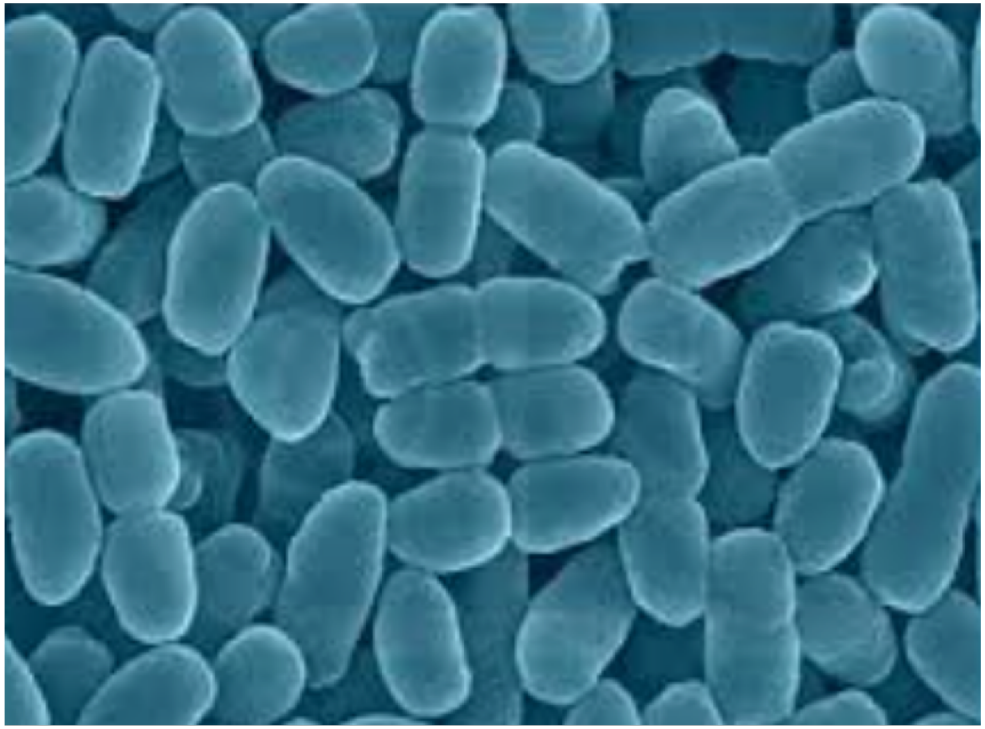


Spirochaetes move by means of an **axial filament**, which resembles a flagellum but is contained under an **external flexible sheath**

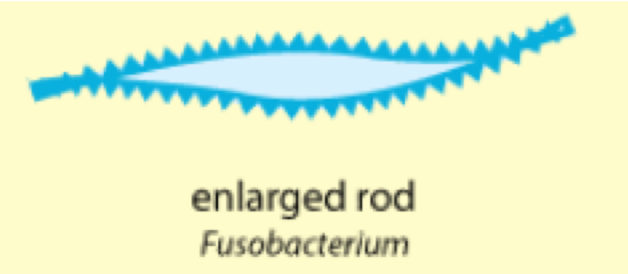
Other forms

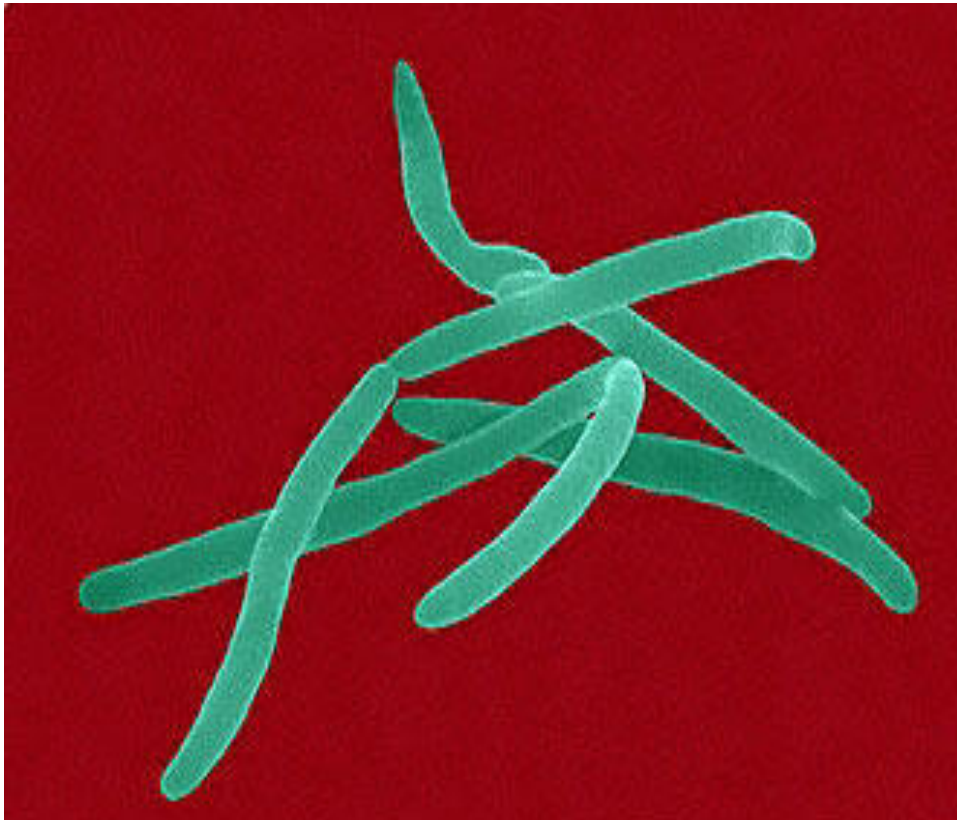
Coccibacillus: Neither rod-shaped nor spherical but in between- they are **short** and **fat**





Fusiform: Tapered at the ends

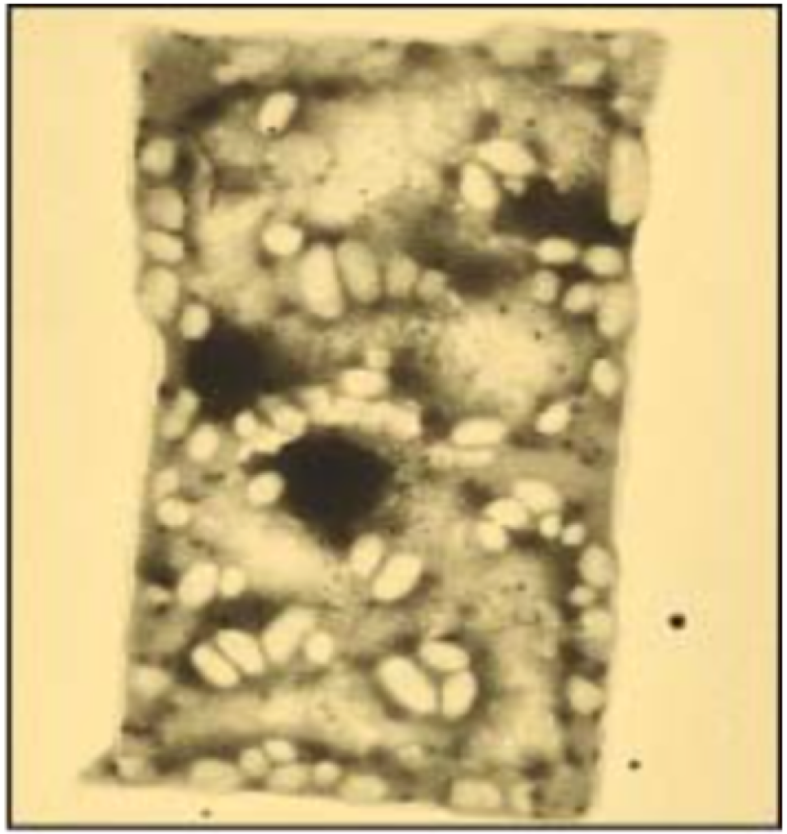




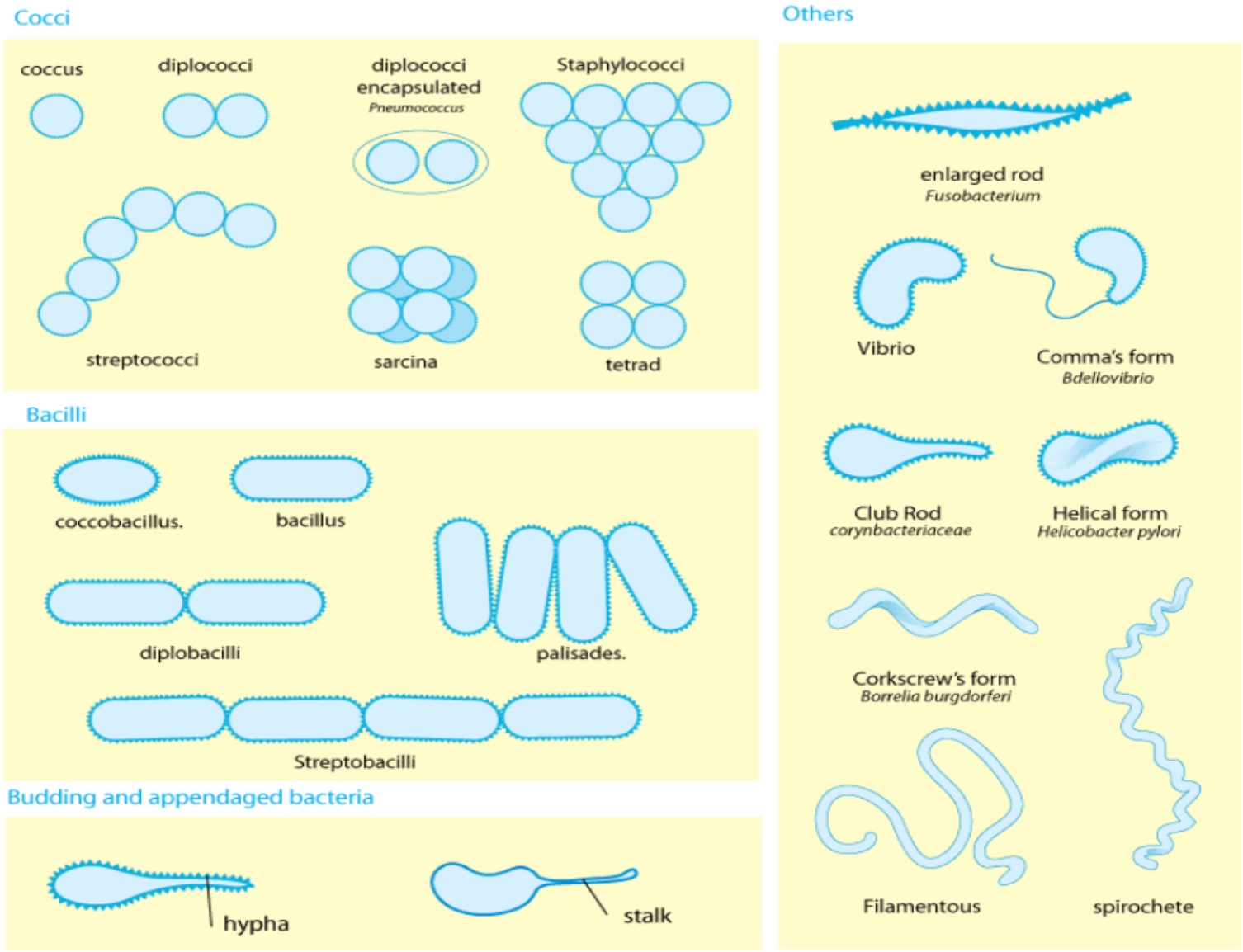
Fusobacterium - Filamentous long threads

Stella/Star-shaped bacteria

Rectangular bacteria



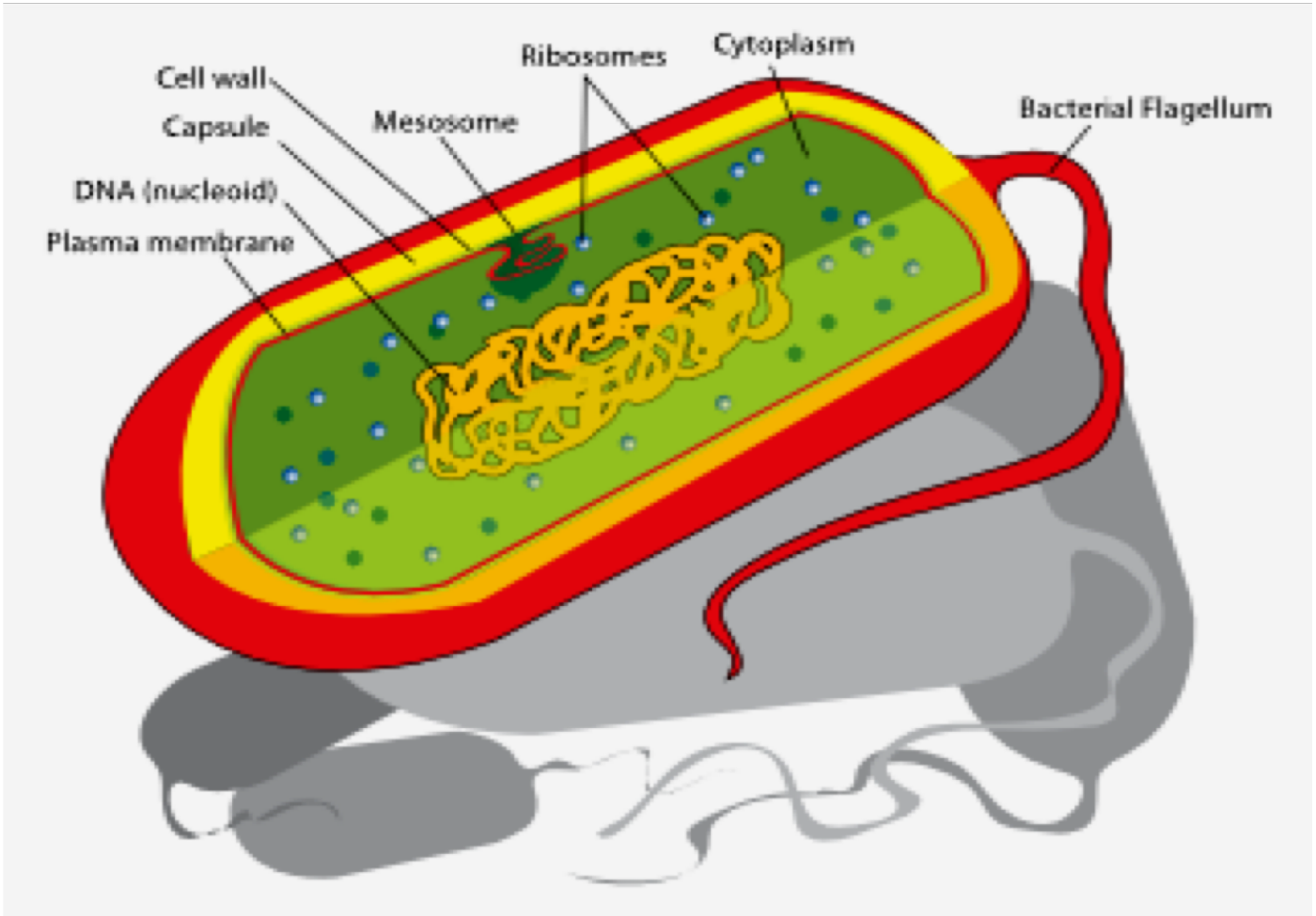
Haloarcula


Cocci
coccus
diplococci
diplococci encapsulated
Pneumococcus
Staphylococci
streptococci
sarcina
tetrad
Bacilli
coccobacillus.
bacillus
diplobacilli
palisades.
Streptobacilli
Budding and appendaged bacteria
hypha
stalk
Others
enlarged rod
Fusobacterium
Vibrio
Comma's form
Bdellovibrio
Club Rod
corynbacteriaceae
Helical form
Helicobacter pylori
Corkscrew's form
Borrelia burgdorferi
Filamentous
spirochete

Arrangements

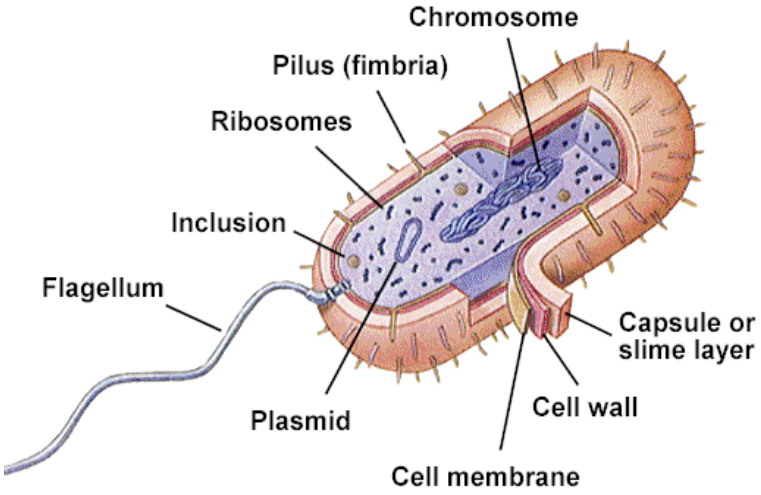
- Generally bacterial shape is determined by **heredity**
- Most bacteria are **Monomorphic** or they maintain a single shape
- However, a number of **environmental** conditions can alter that shape
- If the shape is altered, the identification becomes difficult
- For example the shape of *Rhizobium* and *Corynebacterium* are genetically **Pleomorphic** or can have many shapes.

Typical bacteria CELL structure

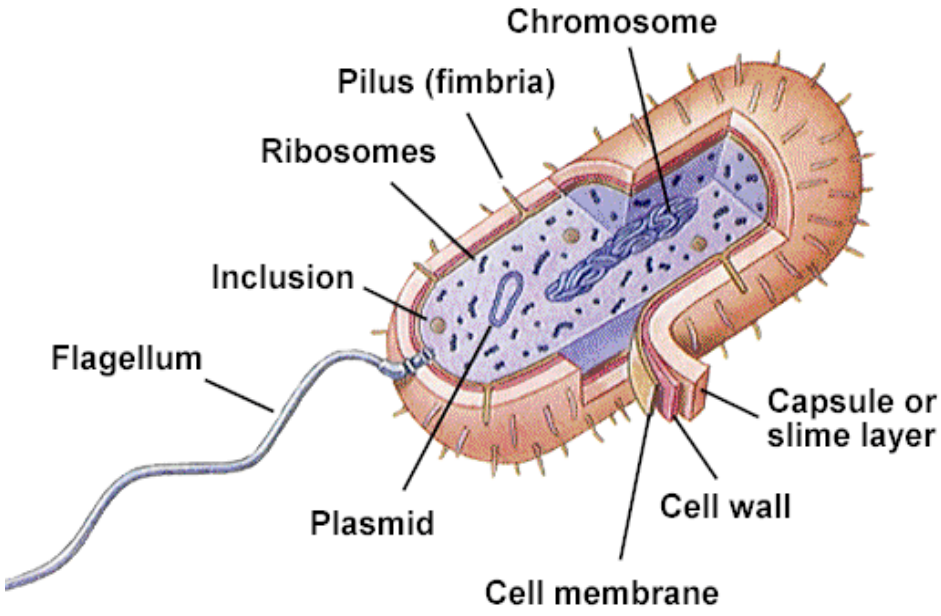


Structure

- Appendages - flagella, pili or fimbriae
- Surface layers - capsule, cell wall, cell membrane
- Cytoplasm - nuclear material, ribosome, mesosome, inclusions etc.
- Special structures - endospore



- Features or structures outside are:
  - Glycocalyx (Capsule)
  - Flagella
  - Axial Filaments
  - Fimbriae and Pili



GLYCOCALYX/CAPSULE

- Consists of **polysaccharide**, **polypeptide** or both
- Viscous (sticky), gelatinous substance and can take the form of either a capsule or a slime layer
- Is often produced from within the cell and excreted outside
- If Glycocalyx is firmly attached to the cell wall it is called a **CAPSULE** (The layer is well organized and not easily washed off)
- But if loosely attached it is called a **SLIME LAYER** (unorganized material that is easily removed)

Importance of capsule

Capsules are important in:

- Determining bacterial **virulence** (Degree to which a bacteria can cause a disease)
- Often protect pathogenic bacteria from **phagocytosis** by the cells of the host

E.g. *Streptococcus pneumoniae* causes pneumonia when the cells are protected by a capsule

Uncapsulated *S. pneumoniae* cells are readily phagocytized and cannot cause pneumonia

Same as *B. anthracis* which causes anthrax when capsulated.

Enables organisms **attach** themselves to various surfaces such as:

- plant roots, human teeth and tissues
- E.g. *Streptococcus mutans* on teeth, *Klebsiella pneumoniae* to the respiratory tract.

FLAGELLA

- Structures on bacteria which enable them to **move** (motility) either in a clockwise or counterclockwise direction
- *Proteus* for e.g. can “swarm” on solid culture media (spread across petri dish)
- Movement of a bacterium towards or away from a stimulus is called a **TAXIS**

- Stimulus can either be chemical (**chemotaxis**) or light (**Phototaxis**)
- Through this receptor, microbes can either be attracted or repelled from a stimulus.
- Flagella is about 12 microns in length
- The major protein component is **flagellin**

- Can be observed by the **hanging drop technique** (bacteria motility test)

***Bacteria have four major arrangements of flagella:

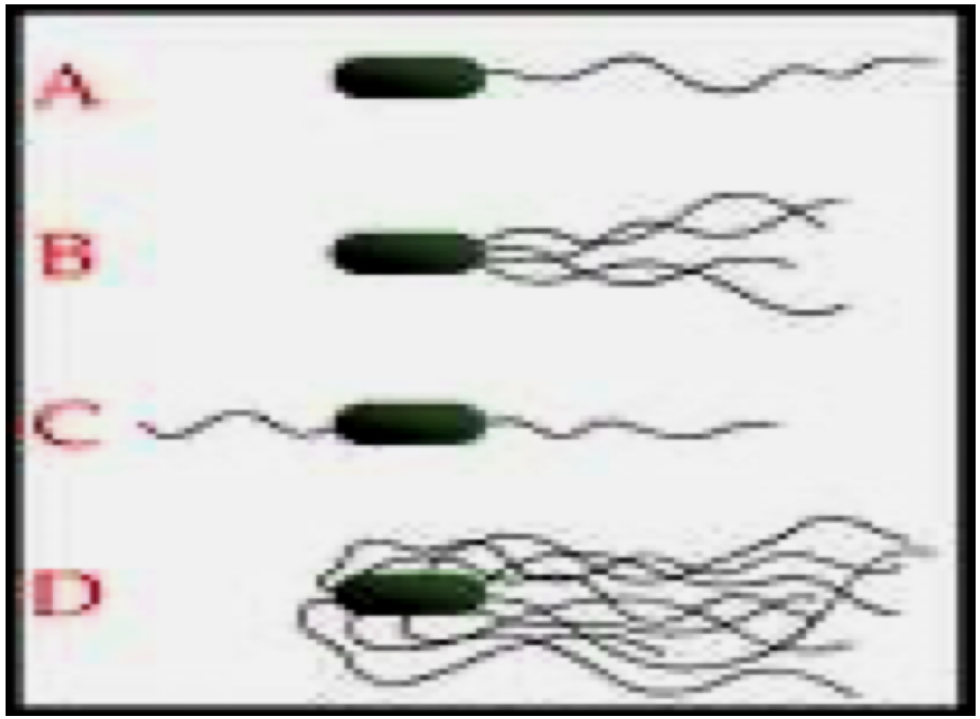
(A) Monotrichous – **single** polar flagellum

(B) Lophotrichous – **two** or **more** flagella at one or both poles of the cell

(C) Amphitrichous - **single** flagellum at each end of the cell

(D) Peritrichous – flagella distributed **over** the entire cell

**Arrangements of flagella

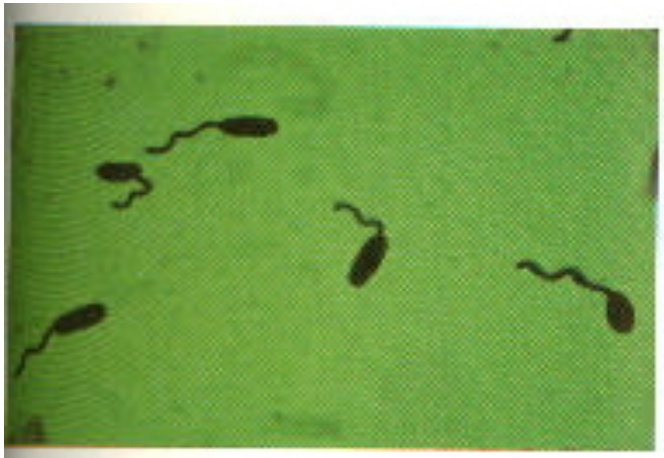


Monotrichous

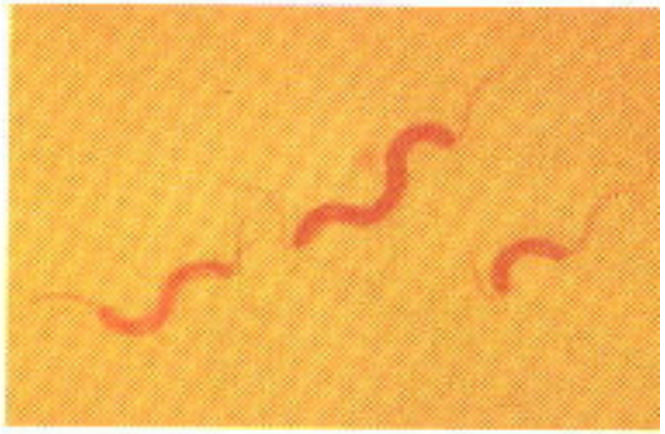
Lophotrichous

Amphitrichous

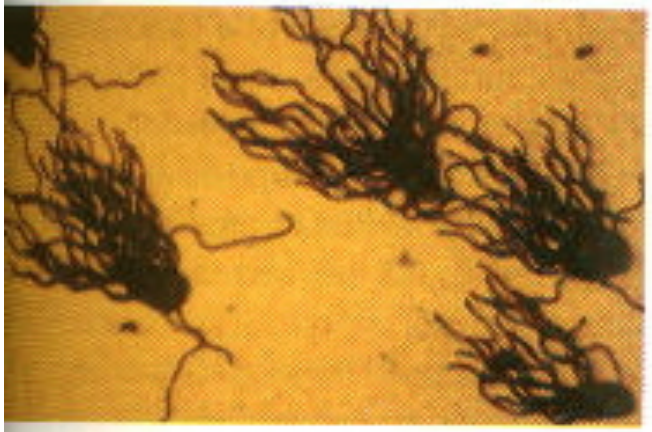
Peritrichous



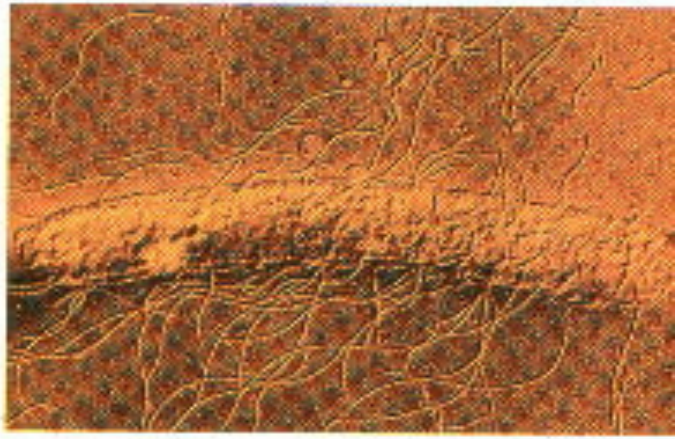
(a)



(b)



(d)



(e)

FIMBRIAE AND PILI

- Are hair-like appendages that are shorter, smaller straighter and thinner than flagella
- Used for **attachment/adhere** rather than for motility
- Mainly found on **Gram –ve** rods
- Originates from the **cytoplasm** and extend outwards from the surfaces Chemical composition confers antigenic specificity.
- Environmental factors such as temp and oxygen tension affect the presence or absence of the pili.
- Pili help in the transfer of DNA from one cell to the other

CELL WALL

- Responsible for the maintenance of the shape of the cell.
- Protects the plasma or cytoplasmic membrane from the surrounding environment.
- Prevents the bacterial cell from rupturing when the osmotic pressure inside the cell is greater than that outside the cell.
- Enables some bacterial species to cause diseases and is the site of action of some antibiotics
- Its composition is used to differentiate between major bacterial types.

General structure of cell wall

- Macromolecular network called **PEPTIDOGLYCAN**
- It is either present singly or in combination with other substances
- Peptidoglycan consists of a repeating disaccharide attached by polypeptides to form a lattice that surrounds and protects the entire cell
- The disaccharide portion is made up of monosaccharides called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) which are related to glucose.
- N-acetylmuramic acid is unique to prokaryotic cell.

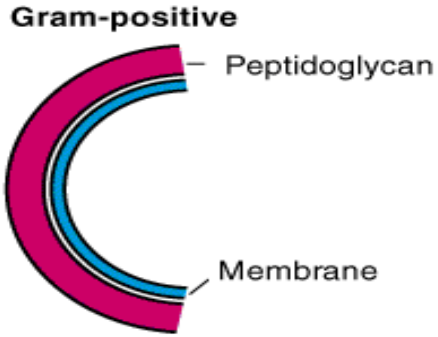
GRAM +ve

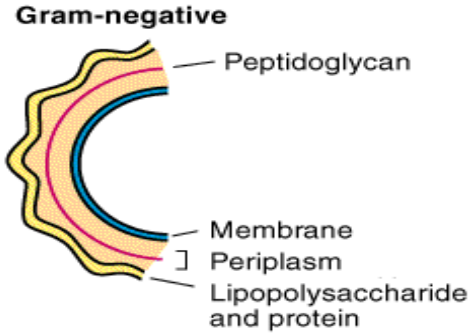
- Many layers of layers
  a thick, rigid structure
- Contain large amounts of teichoic acid, alcohol (glycerol or ribitol) and phosphate

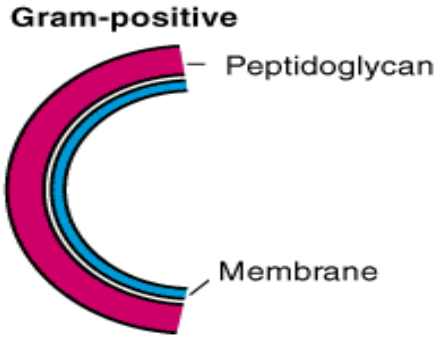
GRAM –ve

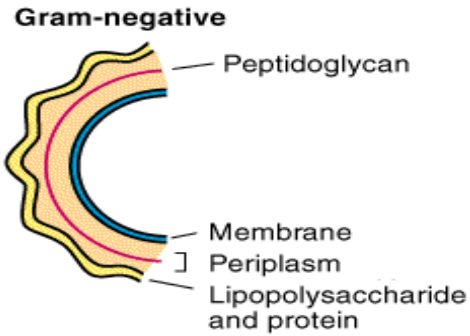
Only one or few thin peptidoglycan forming

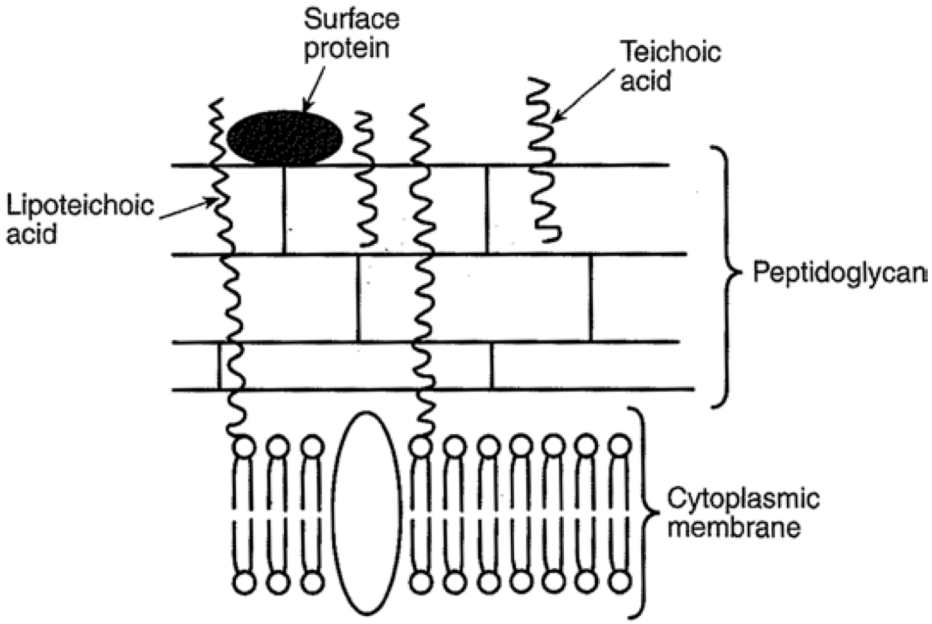
Does not contain teichoic acid

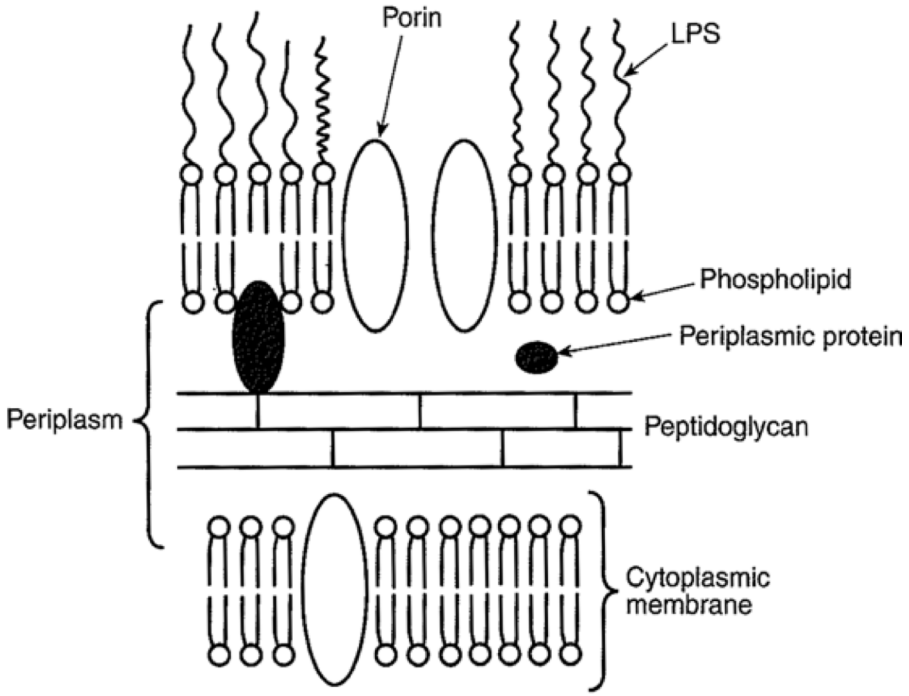




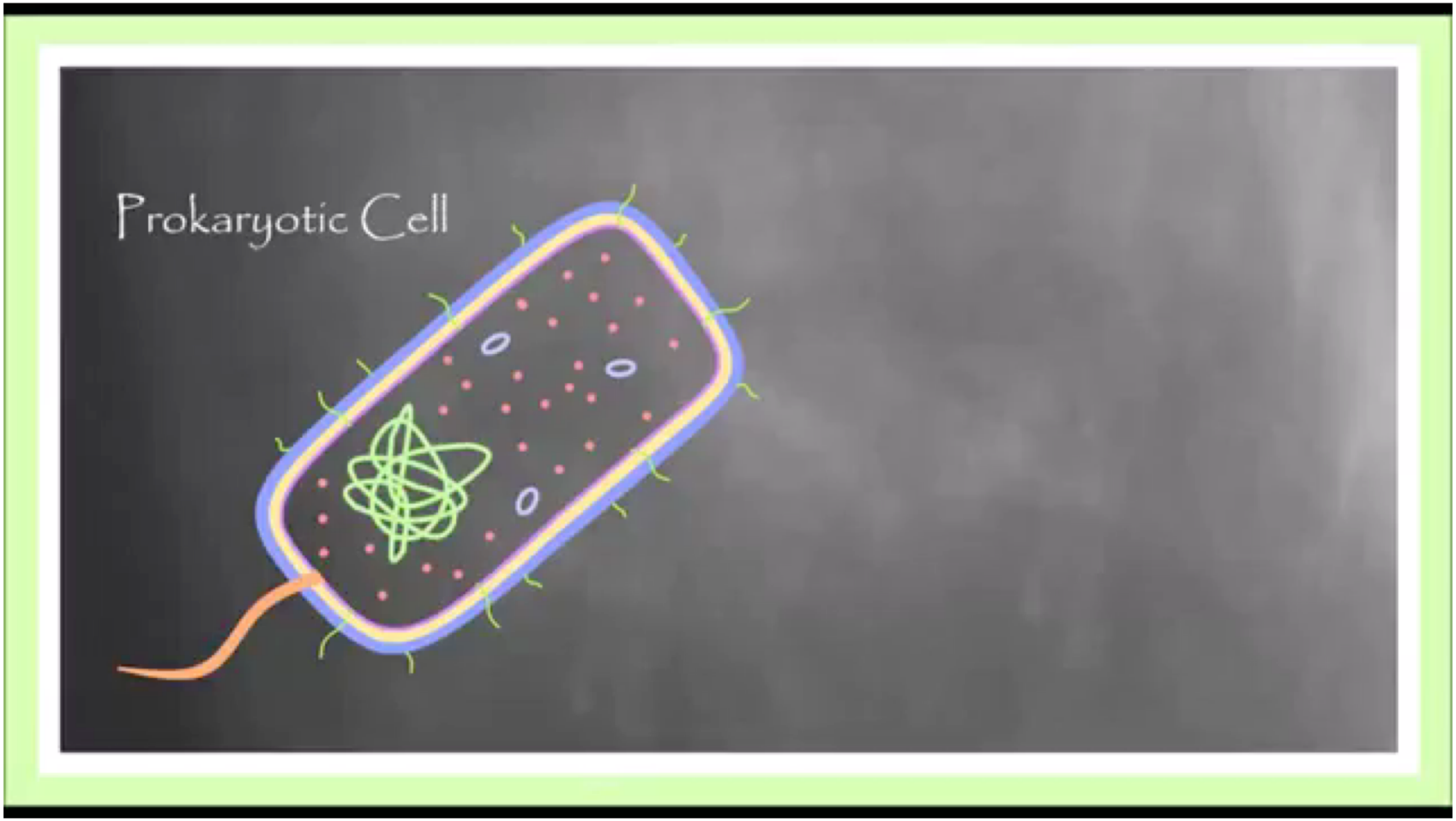

Gram-positive
Peptidoglycan
Membrane


Gram-negative
Peptidoglycan
Membrane
Periplasm
Lipopolysaccharide
and protein


Surface
protein
Teichoic
acid
Lipoteichoic
acid
Peptidoglycan
Cytoplasmic
membrane


Porin
LPS
Phospholipid
Periplasmic protein
Periplasm
Peptidoglycan
Cytoplasmic
membrane

Video



NUTRITION AND GROWTH

NUTRITION AND GROWTH

Nutrition: Process by which chemical substances (nutrients) are acquired from the environment and used (utilized) in cellular activities.

Essential nutrients: Must be provided to an organism

Two categories:

1. Macronutrients: requires in large quantities; play primary roles in cell structure and metabolism eg. Proteins, carbohydrates, lipids
2. Micronutrients: required in small amounts; involved in enzyme function and maintenance of protein structure eg. Manganese, zinc, Sodium

NUTRITION AND GROWTH

- Every organism must find in its environment all of the substances required for energy generation
- Chemicals and elements of the environment that are utilized for bacterial growth are referred to as **NUTRIENTS**
- In the laboratory, bacteria are grown in **CULTURE MEDIA** which are designed to provide all the essential nutrients in solution for bacterial growth

NUTRITION AND GROWTH

- Bacteria are **ubiquitous** (found everywhere)
- They exhibit a wide range of tolerance to the environment
- Obtain energy from an amazing variety of substrates
- Show the most extreme forms of metabolism for any given environmental factor

Chemical analysis of Microbial cytoplasm

- 70% water
- Proteins

96% of cell is composed of **6** key elements

- Carbon
- Hydrogen
- Oxygen
- Phosphorous
- Sulfur/Sulphur
- Nitrogen

OXYGEN REQUIREMENT

- They can be classified based on their **oxygen requirements**
- **Aerobic:**

  Require oxygen to grow
- **Anaerobic**

  Do not require oxygen to grow
- **Microaerophilic**

  Require very little oxygen to grow e.g. (*Campylobacter spp., Helicobacter pylori*)

Obligate aerobes

- Grow **only** in the presence of oxygen (*Mycobacterium tuberculosis* and *Nocardia asteroides,* fungi, algae)

Obligate anaerobes

Do not need or use oxygen to grow (*Actinomyces*, *Bacteroides*, *Clostridium*, *Fusobacterium)*

Oxygen is toxic to them, as it either kills or inhibits their growth

- They may live by fermentation, anaerobic respiration

(the rumen fungi *Neocallimastix*, *Piromonas*, and *Sphaeromonas)*

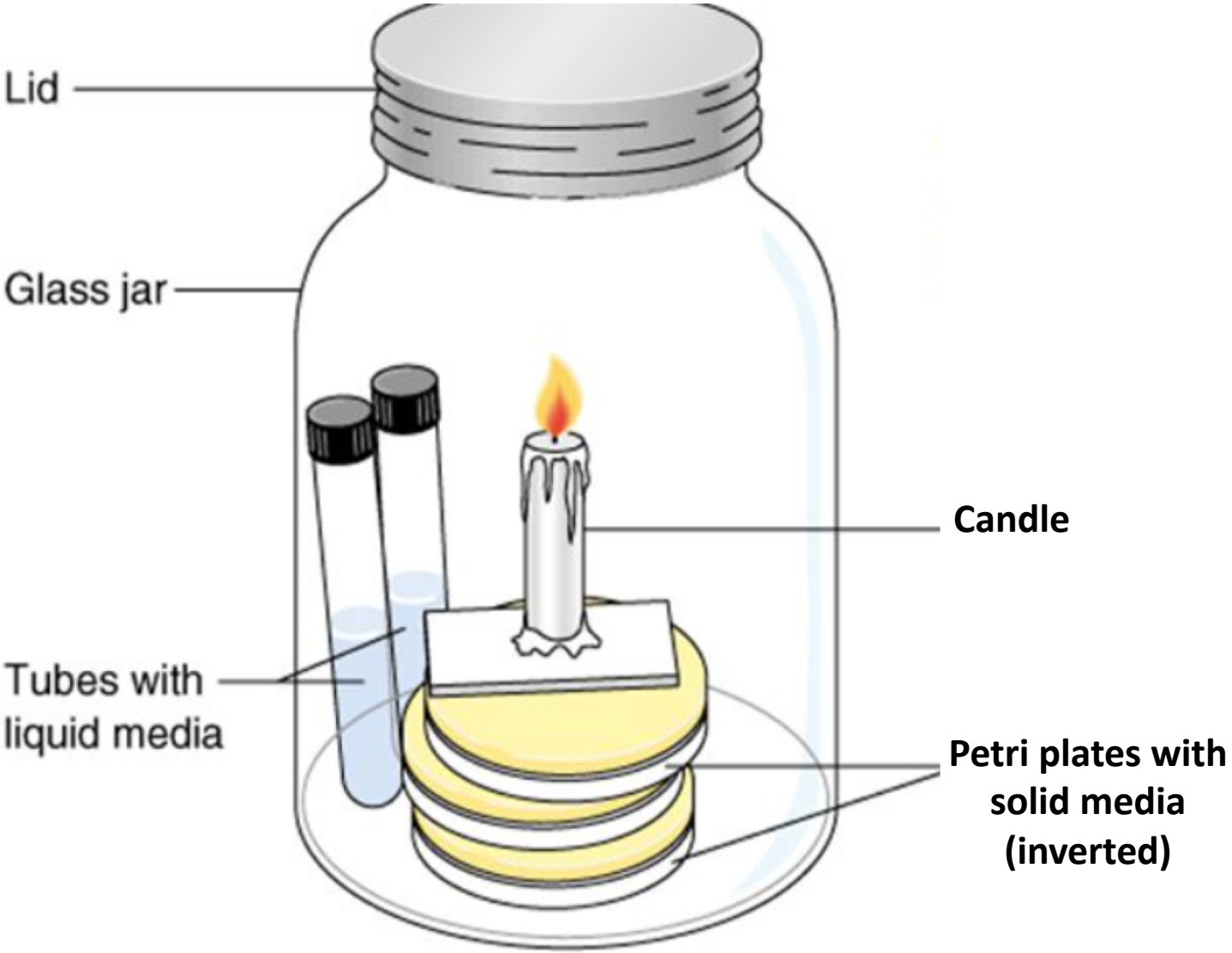
- **Facultative anaerobes** (or **facultative aerobes**) can switch between aerobic and anaerobic types of metabolism
- Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration
- But in the presence of O_2 they switch to aerobic respiration. e.g. Yeast

- *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Listeria* spp.
- Certain eukaryotes are also facultative anaerobes, including fungi such as *Saccharomyces cerevisiae*

- **Aerotolerant anaerobes** are bacteria with an exclusively anaerobic (fermentative) type of metabolism
- They are insensitive to the presence of O_2
- They live by fermentation alone whether or not O_2 is present in their environment e.g. lactic acid bacteria

- **Capnophiles**

  Grow well with low oxygen and high carbon dioxide


Lid
Glass jar
Candle
Tubes with liquid media
Petri plates with solid media (inverted)

CARBON REQUIREMENT

Carbon- can be classified based on their carbon source

- **Heterotrophs**: Obtain carbon in an organic form made by other living organisms
- **Autotrophs**: Use CO_2, an inorganic gas as its carbon source (pure inorganic diets)
- When they can fix carbon dioxide using light, they are called **Photoautotrophs** e.g. Cyanobacteria
- Or from chemical reactions usually oxidations i.e. reduced inorganic molecules, **chemoautotrophs** eg. methanogens

- **Litotrophs -** oxidation of inorganic substances

- **Organotrophs** - oxidation of organic substances

Most growth is heterotrophic and ranges from the use of simple hexoses to utilising complex carbon compounds.

NITROGEN REQUIREMENT

NITROGEN- there are parts of the nitrogen cycle which can only be carried out by bacteria

- **Nitrogen fixation**
- **Nitrification**
- **Denitrification**

- Bacterial nitrogen metabolism ranges from the use of molecular nitrogen (N fixation) through to Proteolysis as found in gas gangrene.

(A type of gangrene that arises from dirty, lacerated wounds infected by anaerobes e.g. *Clostridium*).

TEMPERATURE REQUIREMENT

- Minimum temperature - Lowest temp that permits a microbe's growth and metabolism
- Maximum temperature - Highest temp that permits a microbe's growth and metabolism
- Optimum temperature - Promotes the fastest rate of growth and metabolism

TEMPERATURE REQUIREMENT

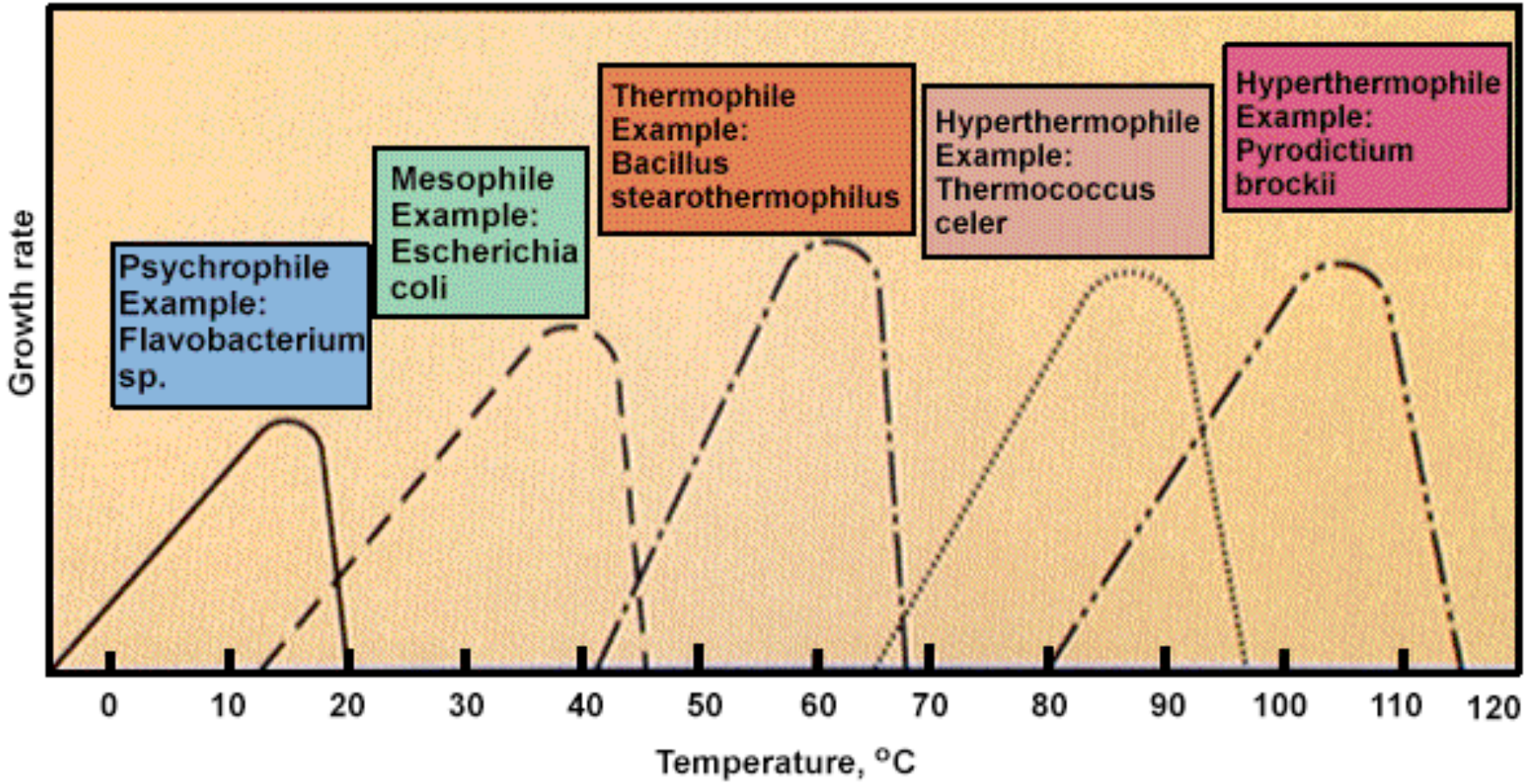
Temperature-

- **Psychrotrophic/Psychrophiles**: Bacteria can grow in refrigerators (*Listeria*, *Proteus*) – optimum temp below 15^{o}C (capable of growing at 0^{o}C)

- **Mesophilic/Mesophiles**: Most soil and water bacteria are with an optimum temperature of 25^{o}C and many pathogens of man grow best at 37^{o}C (20-40^{o}C)

- **Thermophilic/Thermophiles:** Bacteria are found in hot springs and volcanic vents (Temp above 45^{o}C)

Ecological groups by temperature of adaptation



Effects of pH

- Obligate acidophiles – grow at extreme acid pH

- Alkalinophiles – grow at extreme alkaline pH

Osmotic Pressure

- Halophiles - Require a high concentration of salt

(*Halobacterium*)

- Osmotolerant - Do not require high concentration of solute but can tolerate it when it occurs

Most microbes exist under hypotonic or isotonic conditions

End of class