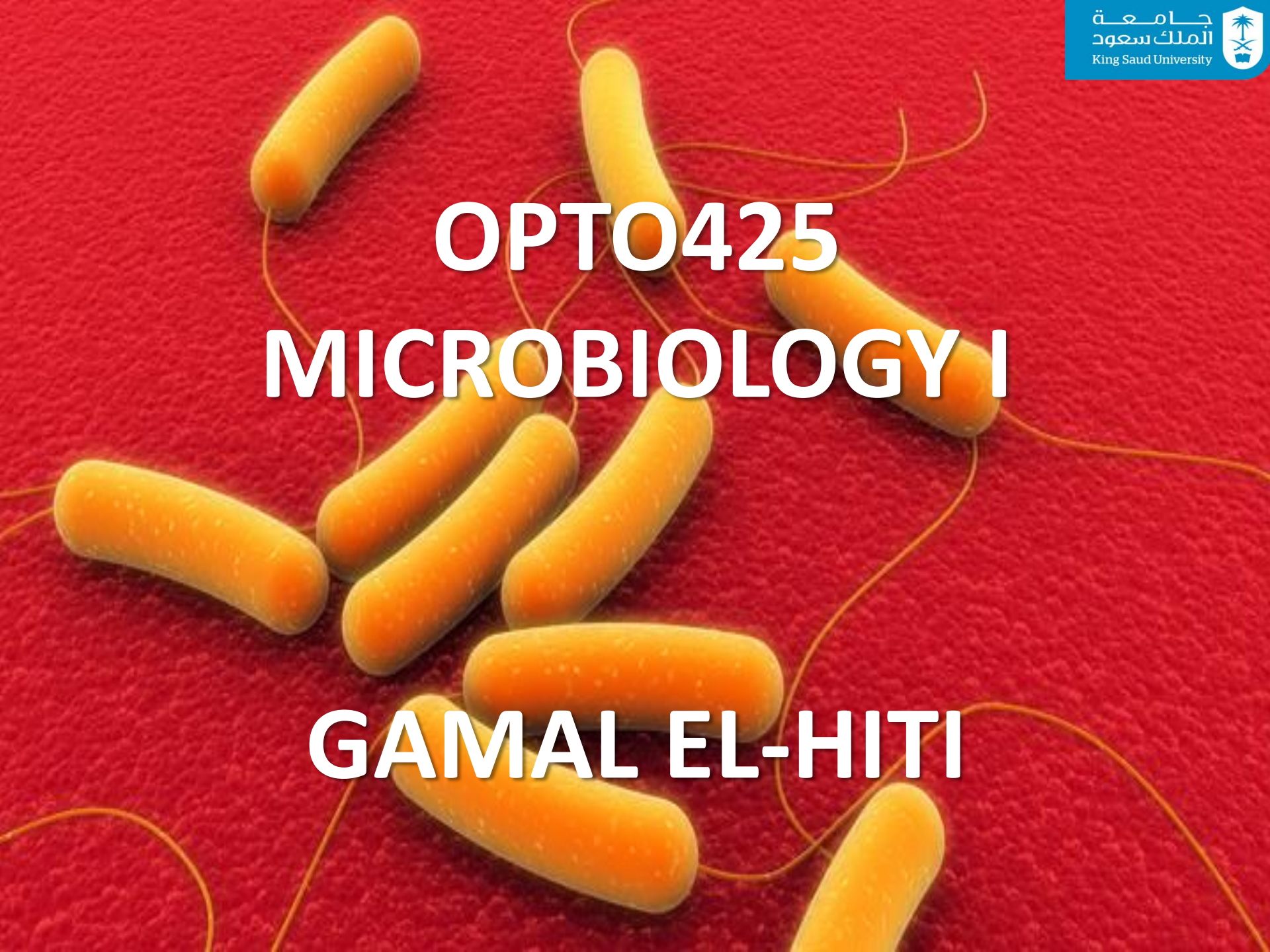


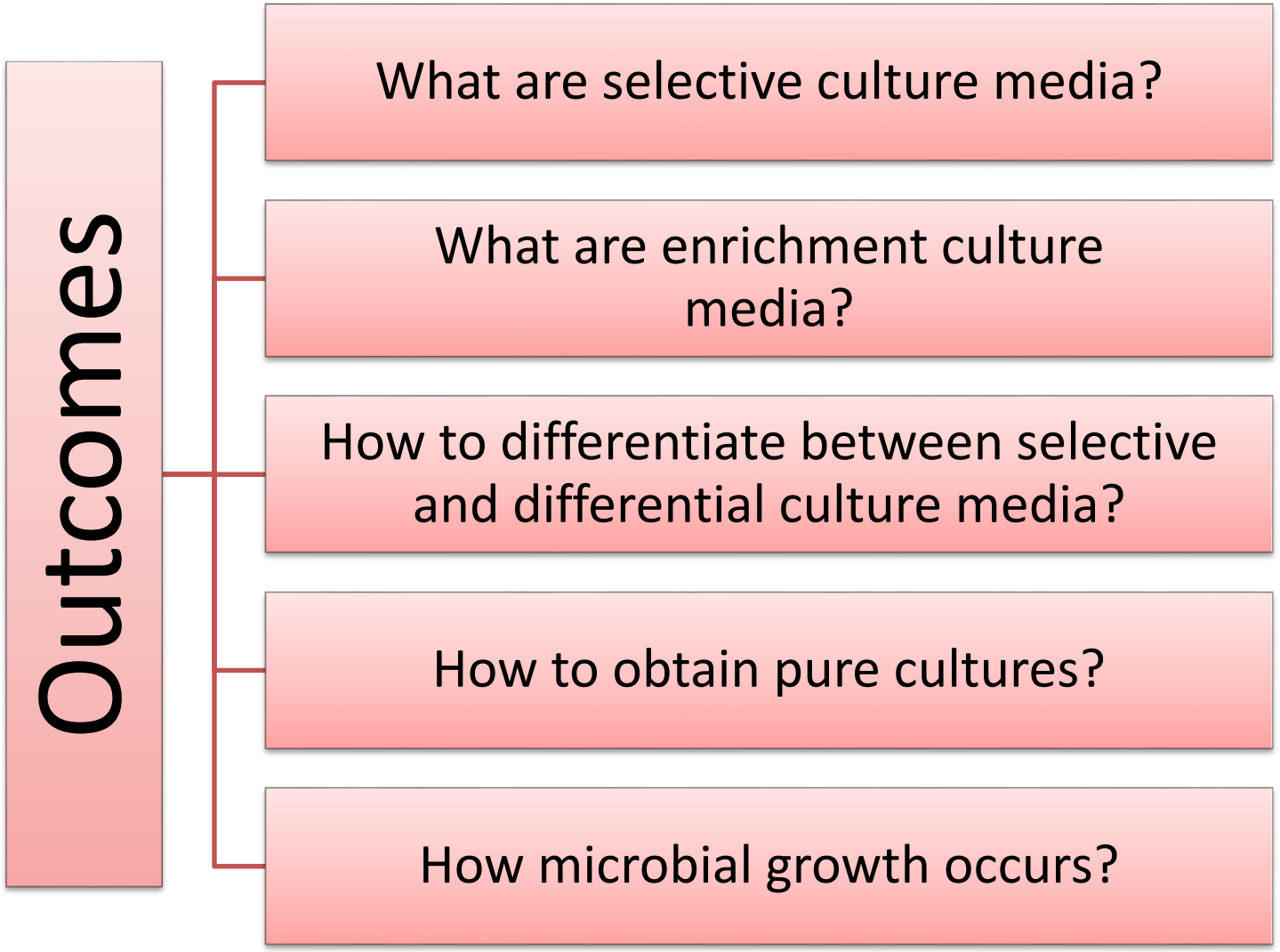
OPTO425
MICROBIOLOGY I

GAMAL EL-HITI

Culture Media

OPTO425
Lecture Eight

Learning Outcomes



Culture Media

- Culture
- Microbes that grow and multiply in or on a culture medium.
- Culture Medium
- Nutrient material prepared for microbial growth in the laboratory.
- Requirements
- Must be sterile.
- Contain appropriate nutrients.
- Incubated at appropriate temperature.

Culture Media

- Solid Media
- Nutrient material that contains a solidifying agent (plates, slants, deeps, *etc.*).
- Agar is the most common solidifier.
- Unique Properties of Agar
- Melts above 95 °C.
- Once melted, does not solidify until 40 °C.
- Cannot be degraded by most bacteria.
- Polysaccharide made by red algae.
- Originally used as food thickener.

Culture Media

- Chemically Defined Media
- Nutrient material whose exact chemical composition is known.
- For chemoheterotrophs, must contain organic source of carbon and energy (*e.g*. glucose, starch, *etc.).*
- May contain amino acids, vitamins and other building blocks that microbe need.
- Not widely used.
- Expensive.

Culture Media

- Complex Media
- Complex media contain nutrient materials whose exact chemical composition is not known.
- Complex media are widely used for heterotrophic bacteria and fungi.
- Complex media are made of extracts from yeast, meat, plants, protein digests, *etc*.
- Complex media composition may vary slightly from batch to batch.

Culture Media

- Energy, carbon, nitrogen and sulfur requirements are primarily met by protein fragments (peptones).
- Peptone is a mixture of polypeptides and amino acids formed by the partial hydrolysis of protein. Protein is a polypeptide that contains more than 50 amino acids.
- Two forms of complex media are common:
- Nutrient broth: liquid media.
- Nutrient agar: solid media.

Culture Media

- Anaerobic Growth Media
- Used to grow anaerobes that might be killed by oxygen.
- Reducing media.
- Contain ingredients that chemically combine with oxygen and remove it.
- Example: sodium thioglycolate
- Tubes are heated shortly before use to drive off oxygen.
- Plates grown in oxygen free containers.

Anaerobic Culture System

- Reducing media chemically remove oxygen (O_2).
- Reducing media might interfere with the growth of anaerobes.
- Oxidation and reduction are symmetric processes, always occurring together, there is always an oxidizing agent and a reducing agent in the reaction.
- Thioglycolate combines with dissolved O_2 to deplete in media.

Anaerobic Culture System

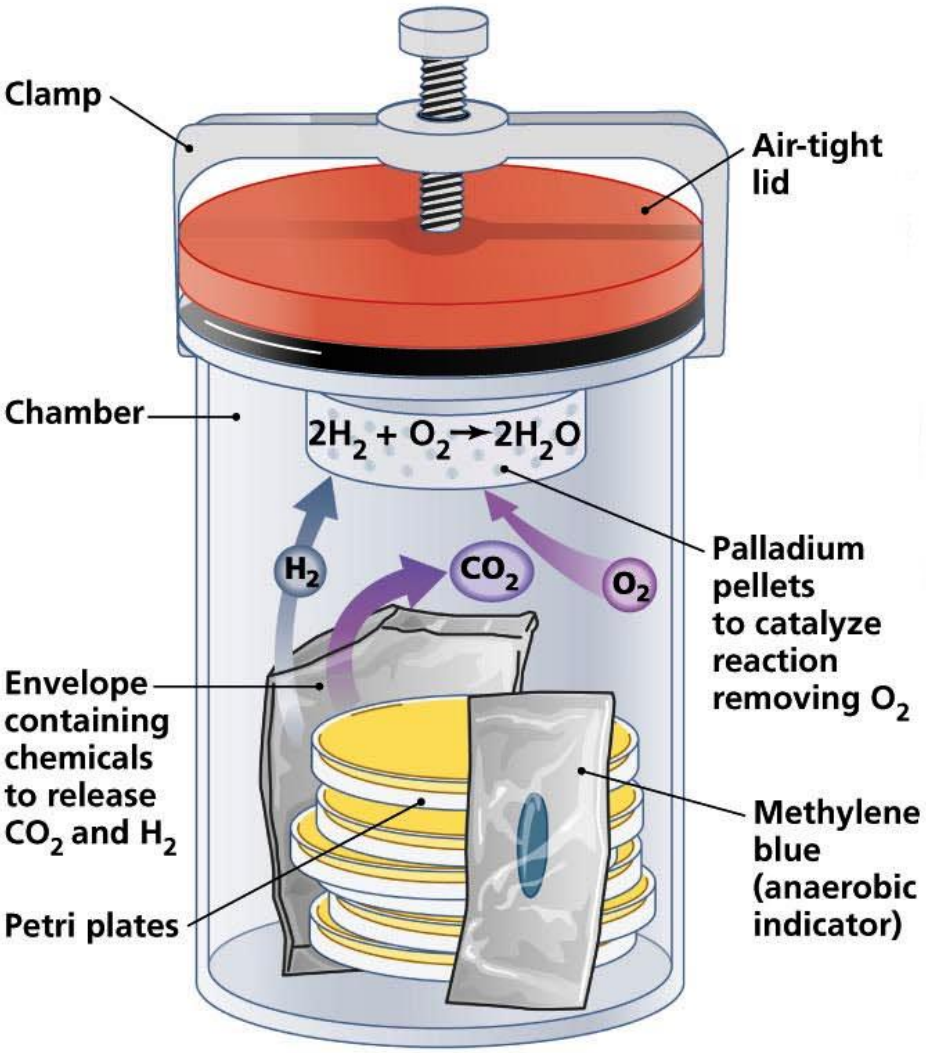
- Petri plates can be incubated in an anaerobic jar or anaerobic chamber.
- Sodium bicarbonate ($NaHCO_3$) and sodium borohydride ($NaBH_4$) mixed with a small amount of water produce CO_2 and H^+.

$$NaHCO_3 + H_2O \longrightarrow H_2CO_3 + OH^- + Na^+$$

$$H_2CO_3 \longrightarrow CO_2 + H_2O$$

- A palladium catalyst and the H^+ (acid) removes O_2.

Anaerobic Culture System



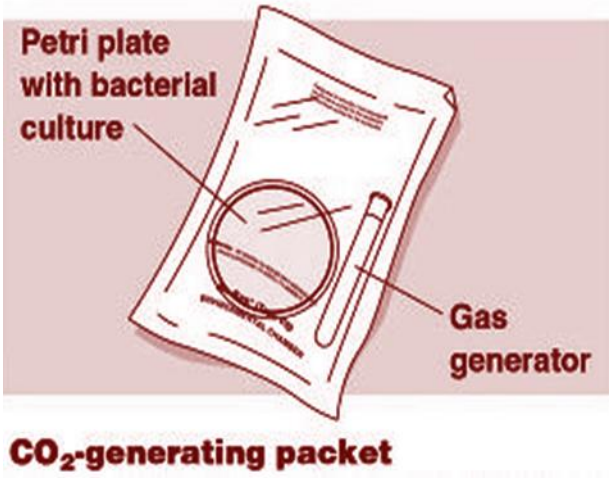
Anaerobic Culture System

Special Culture Techniques

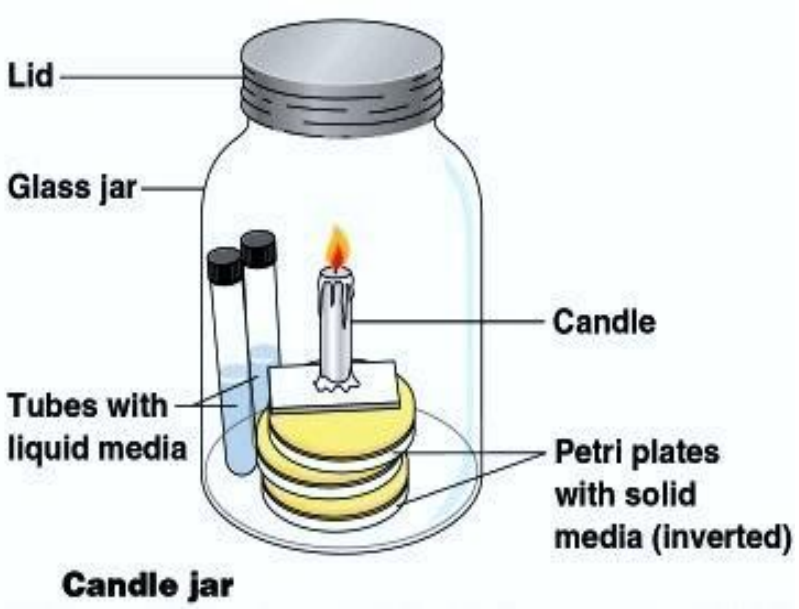
- Some parasitic and fastidious bacteria must be cultured in living animals or in cell cultures.
- Such bacteria won't grow on artificial media.
- CO_2 incubators or candle jars are used to grow bacteria requiring an increased CO_2 concentration (capnophiles).
- Low O_2 and high CO_2 concentrations mimic conditions found in the intestinal tract.

Special Culture Techniques

- The packet consists of a bag containing a petri plate and a CO_2 gas generator.
- The candle jar contains the plates and lighted candle and the jar is sealed.



CO_2-generating packet



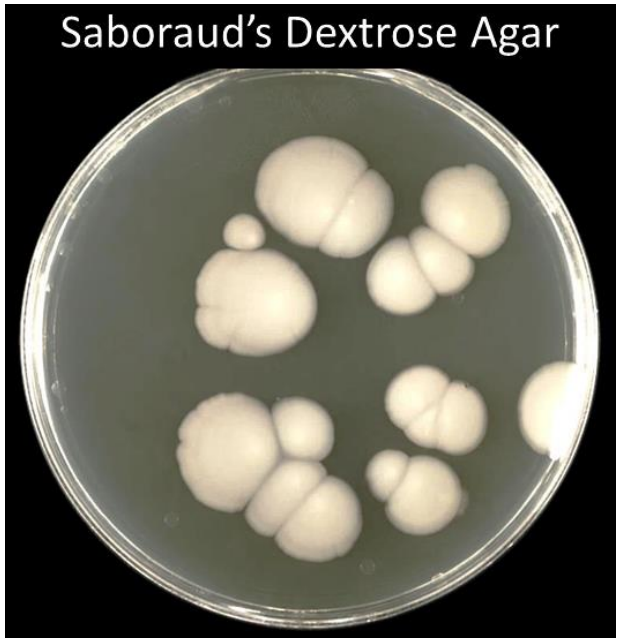
Candle jar

Special Culture Techniques

- CO_2 incubators allow adjustment of CO_2 concentrations to lower or higher than atmospheric concentration when needed by certain aerobic bacteria.
- Candle jars increase the carbon dioxide (CO_2) concentrations and still leave some oxygen (O_2) for the growth of aerobic capnophiles.
- Gas packs can generate CO_2.
- Gas packs are generally used instead of candle jars.

Selective Culture Techniques

- Selective Media
- Used to suppress the growth of unwanted bacteria and encourage the growth of desired microbes.
- Saboraud's Dextrose Agar
- It contains peptones (tetrapeptides).
- The pH of 5.6 discourages bacterial growth.
- This type of agar is used to isolate fungi.



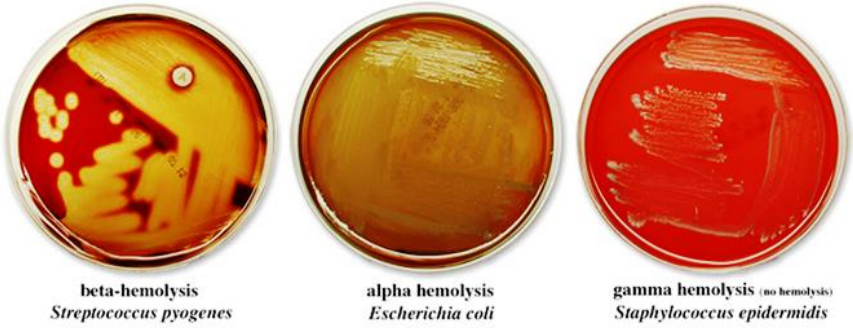
Selective Culture Techniques

- Brilliant Green Agar
- The medium contains yeast extract, NaCl, lactose, sucrose, brilliant green, phenol red, agar and enzymes.
- Green dye selectively inhibits Gram-positive bacteria.
- It is used for the selective isolation of Gram-negative *Salmonella spp*.


Brilliant Green Agar

Culture Media

- Differential Media
- It is used to distinguish colonies of a desired microorganism.
- Blood Agar (sheep's blood)
- It is known as sheep's blood.
- It is used to distinguish bacteria that destroy red blood cells (hemolysis).
- Hemolysis appears as an area of clearing around colony.



Blood Agar

Culture Media

- Both Selective and Differential Media
- Used both to distinguish colonies of a desired microorganism, and inhibit the growth of other microbes.
- Mannitol Salt Agar
- Used to distinguish and select *Staphylococcus aureus*.
- Mannitol fermenter on left (+).
- Non-mannitol fermenter on right (–).
- The pH indicator changes color when mannitol is fermented to acid.

Culture Media

- High salt (NaCl, 7.5%) in mannitol salt agar discourages growth of other organisms.
- MacConkey Agar
- Used to distinguish and select *Salmonella*.
- Bile salts and crystal violet to discourage growth of Gram-positive bacteria.
- Lactose to indicate lactose fermenters (pink colonies) *vs*. non-lactose fermenters (colorless colonies).

MacConkey agar with LF and non-LF colonies

Culture Media

- Enrichment Culture
- It is used to favor the growth of a microbe that might be found in very small numbers.
- Unlike selective medium, does not necessarily suppress the growth of other microorganisms.
- Mainly used for fecal and soil samples.
- After incubation in enrichment medium, greater numbers of microbes obtained and the likelihood of positive identification could increase.

Pure Cultures

- Pure Culture
- Pure culture consist of the descendants of a single cell.
- *i.e.* Contains a single microbial species.
- Most clinical and environmental specimens contain several different microorganisms.
- To obtain a pure culture, individual organisms must be isolated.
- The streak plate is the most common method of isolation.

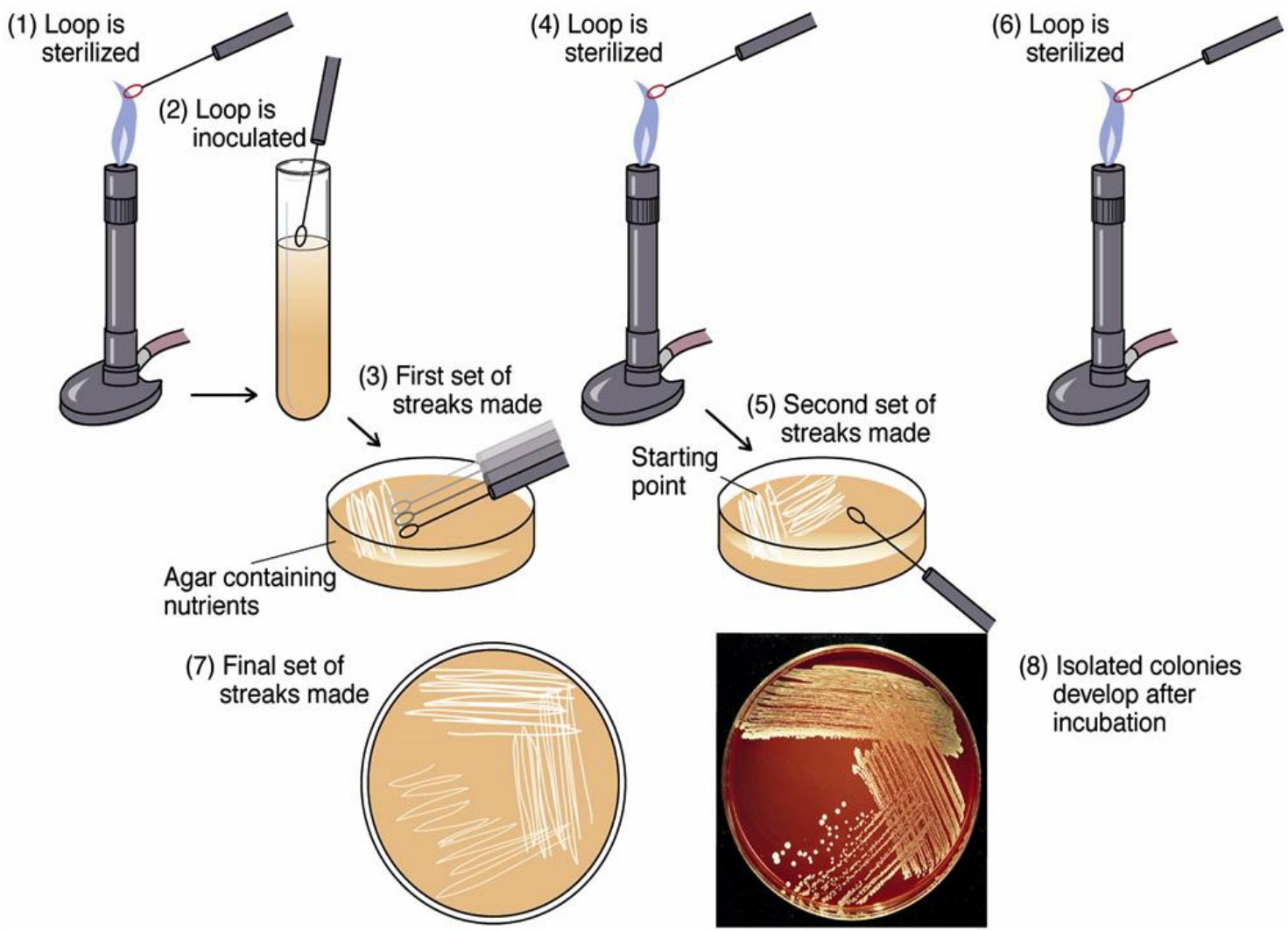
Pure Cultures

- Streaking technique is a rapid and simple process to produce pure cultures.
- The streaking is done using a sterile loop.
- The inoculation loop is first sterilized by passing it through a flame.
- When the loop is cool, it is dipped into a patient specimen containing bacteria.
- The inoculation loop is dragged across the surface of the agar back and forth in a zigzag motion until approximately 30% of the plate has been covered.

Pure Cultures

- The loop then is re-sterilized and the plate is turned 90 degrees.
- Starting in the previously streaked section, the loop is dragged through it two to three times in a zigzag pattern.
- The procedure is then repeated but to not touch the previously streaked sectors.
- The second section shows less growth, while the final section shows the least growth and many isolated colonies.

Pure Cultures



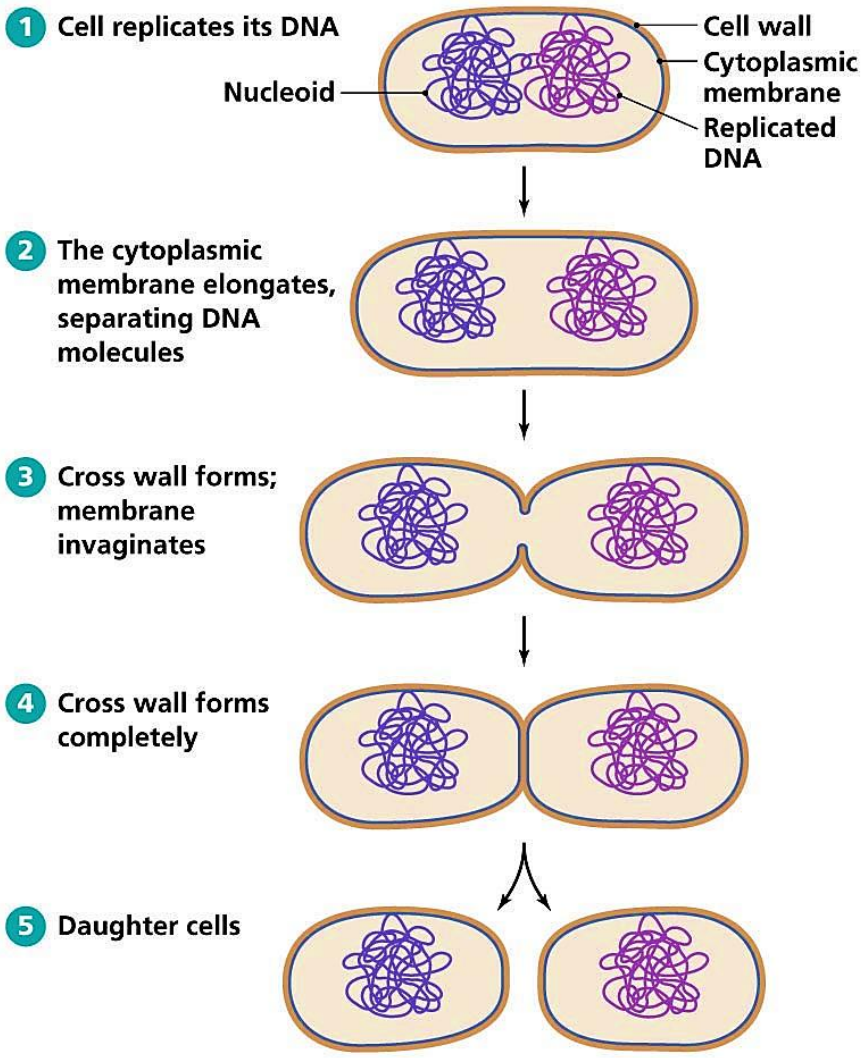
Bacterial Growth

- Bacterial division is the asexual reproduction of bacteria cells.
- It is the cell division of a bacterium cell into two daughter cells.
- Occurs mainly by binary fission.
- A few bacterial species reproduce by alternative means such as budding (yeast) and fragmentation.
- Generation time is time required for a cell to divide and its population to double.

Bacterial Growth

- The bacterial generation time varies considerably from one bacterium to another.
- The growth of a bacterial population occurs in a geometric manner.
- One cell becomes 2 cells (first generation), then 4 cells, then 8 cells, then 16, then 32 and so forth.
- *E. coli* divides every 20 minutes, *i.e.* 20 generations every 7 h (1,000,000 cells).
- Most bacteria divide every 1 to 3 hours.
- Some require over 24 hours to divide.

Binary Fission

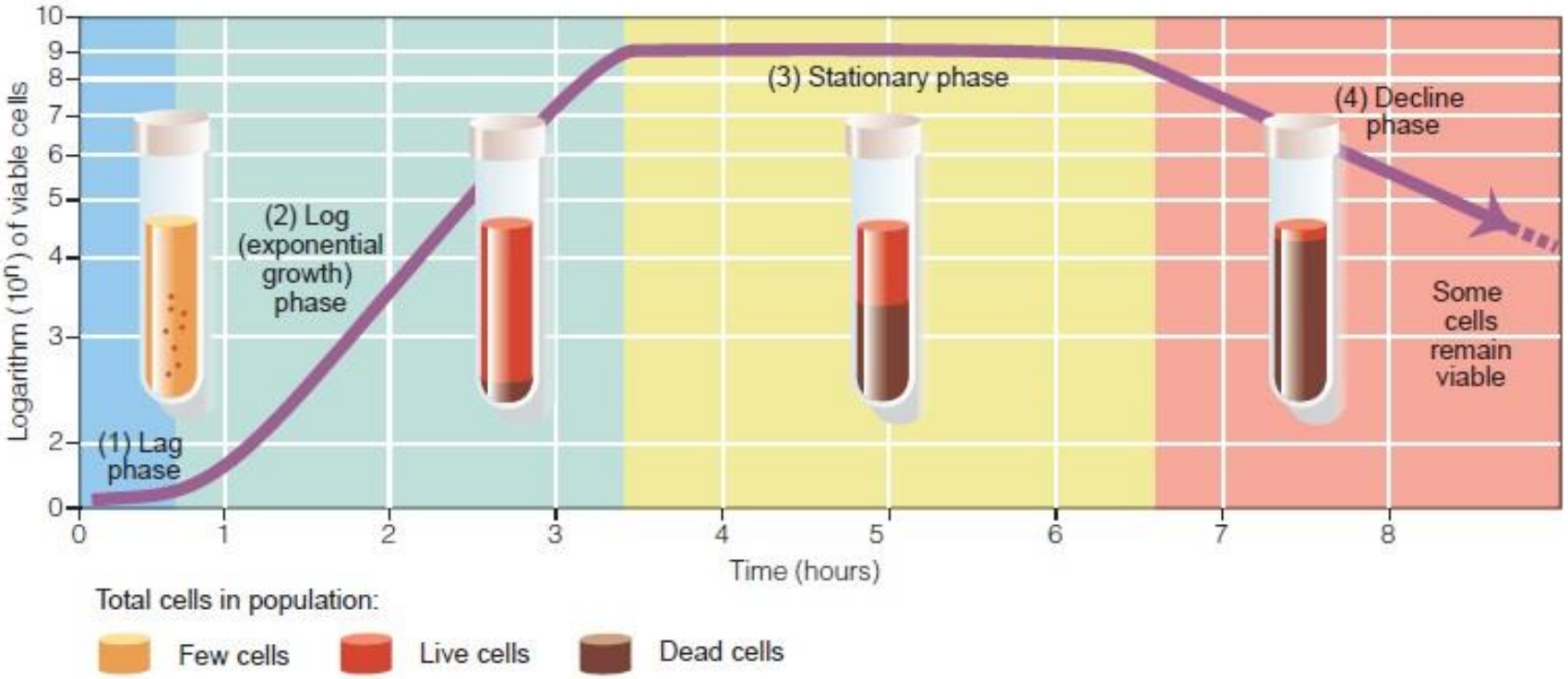


Bacterial Growth

- We can express the number of cells in a bacterial generation as 2^n, where n is the number of doublings that have occurred.
- The superscript indicates the generation, *e.g.* 2^5 means 5 generations.

Arithmetic Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Bacterial Growth

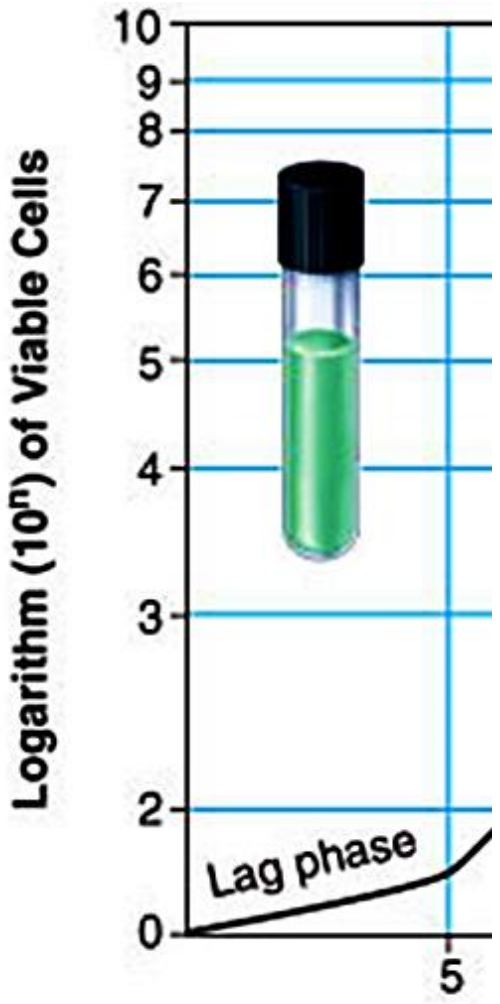


- Standard growth curve shows the growth of a pure culture of bacterial cells over time.
- The cells are incubated from a small amount of bacteria.

Bacterial Growth Curve

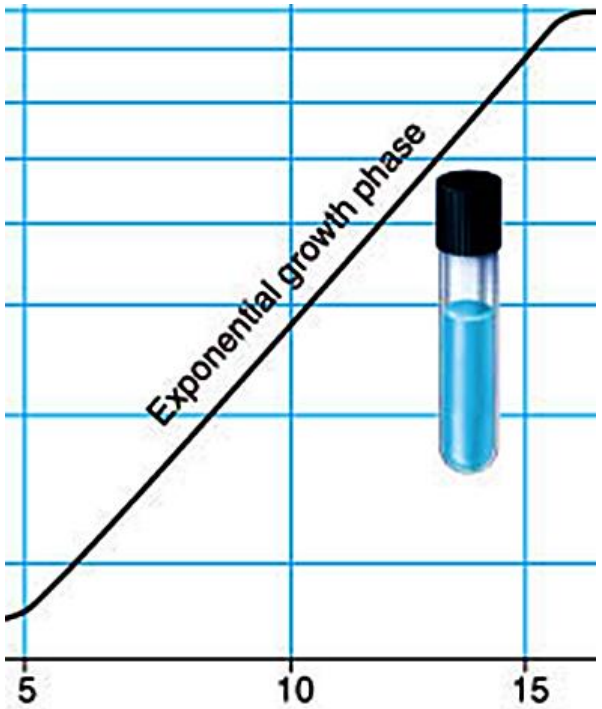
1. Lag Phase

- Period of adjustment to new conditions.
- Little or no cell division occurs, population size doesn't increase.
- Phase of intense metabolic activity, in which individual organisms grow in size.
- May last from one hour to several days.



Phases of Growth

2. Log Phase

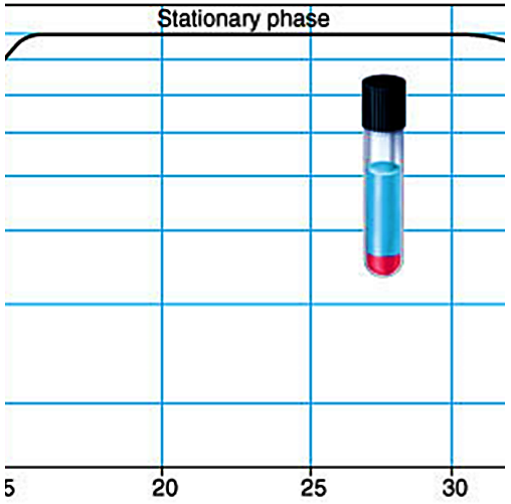


- Cells begin to divide and generation time reaches a constant minimum.
- Period of most rapid growth.
- Number of cells produced > number of cells died.
- Cells are at highest metabolic activity.
- Cells are most susceptible to adverse environmental factors at this stage (*e.g.* radiation and antibiotics).

Phases of Growth

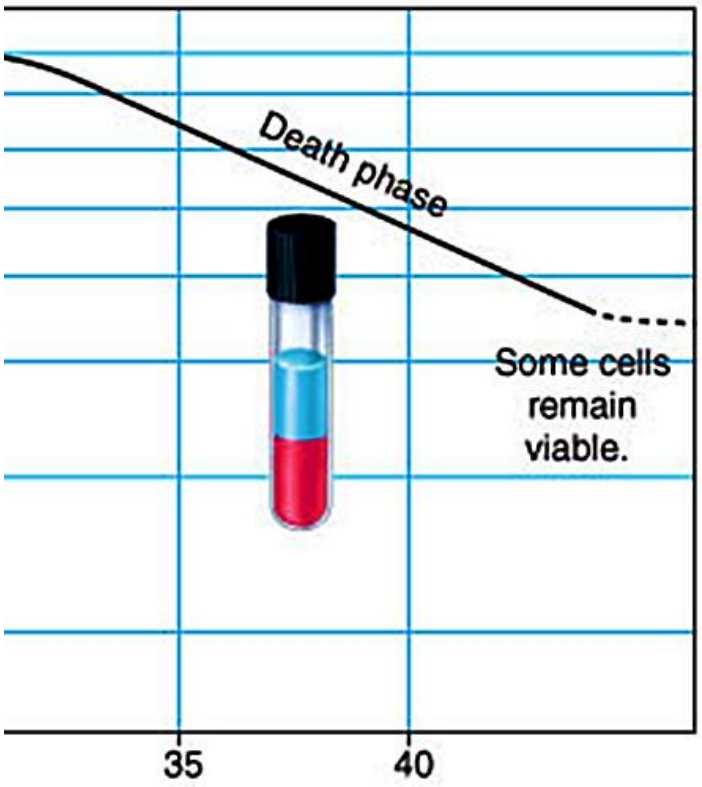
3. Stationary Phase

- Population size begins to stabilize.
- Number of cells produced = Number died.
- Overall cell number does not increase.
- Cell division begins to slow down.
- Factors that slow down microbial growth:
  - Accumulation of toxic waste materials.
  - Acidic pH of media.
  - Limited nutrients.
  - Insufficient oxygen supply.



Phases of Growth

4. Death or Decline Phase



- Population size begins to decrease.
- Number of cells dying are > number of cells produced.
- Cell number decreases at a logarithmic rate.
- Cells lose their ability to divide.
- A few cells may remain alive for a long period of time.