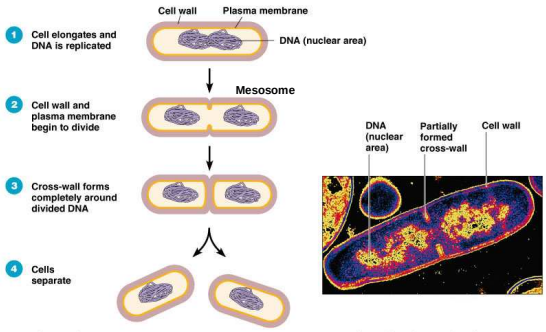
# Microbial Growth

BIO162
Microbiology for Allied Health

## Binary Fission



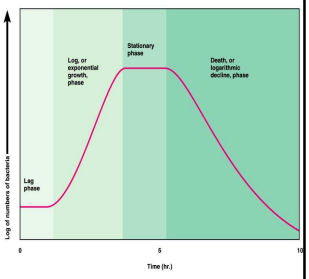

(a) A diagram of the sequence of cell division.




(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

## Bacteria Growth in vitro

- **Binary fission**
  - Splitting parent cell to form two similar-sized daughter cells to increase number of cells
- **Generation time**
  - Duration of each division
  - Determined by type of bacteria
  - Example: *E. coli* (20 min)



## The Requirements for Growth: Chemical Requirements

- **Nutrition**
  - taking in **chemicals**, assimilating them and extracting energy
  - used in metabolism and growth
- **Water**
  - Requirement for all living cells (70-90% water)
  - Bacterial endospores and protozoa cysts can survive in low moisture

## The Requirements for Growth: Chemical Requirements

**Macronutrients**

- required in large amounts
- play role in cell structure and metabolism
- Examples: Carbon, hydrogen, oxygen, nitrogen, phosphate, sulphur

**Micronutrients**

- required in trace amounts
- involved in enzyme function and protein structure
- Examples: zinc, copper, iron
- Present in tap water and distilled water

## The Requirements for Growth: Chemical Requirements

**Growth factors**

- Organic compounds that cannot be synthesized by bacteria
- must be provided as a nutrient; obtained from the environment
- Some bacteria are “fastidious”
- examples: amino acids, purines & pyrimidines (DNA components)

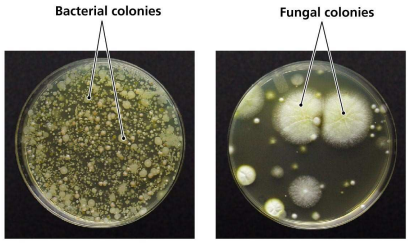
## Culturing bacteria *in vitro*

- *in vitro* – (*latin*, in glass) An *in vitro* biological study is one which is carried out in isolation from a living organism
- **Obligate intracellular pathogens** need to be cultured in chick embryos, lab animals or animal/human cell cultures
- Fungi require different culture medium than bacteria. Low pH and anti-bacterial agents usually added to prevent growth of bacteria in medium for fungal growth e.g. Sabouraud's agar

## Culture media

- Defined medium – exact ingredient is known
- Complex medium – exact content unknown; digested extract of organs or cells; support growth of many fastidious microbes
  - Enriched medium – extra nutrients to promote growth of certain microbes. e.g. chocolate agar (haemoglobin) for *N. gonorrhoeae*
- Selective medium – added inhibitors to discourage growth of certain microbes. e.g. mannitol salt (MSA) agar for salt-tolerant microbes (*Staphylococcus carnosus*)

## Use of selective medium



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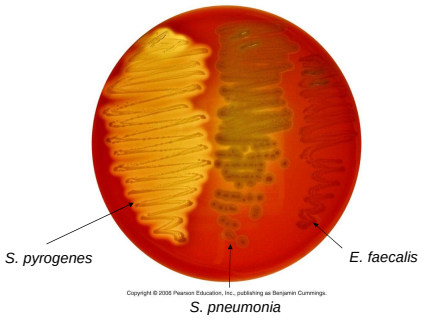
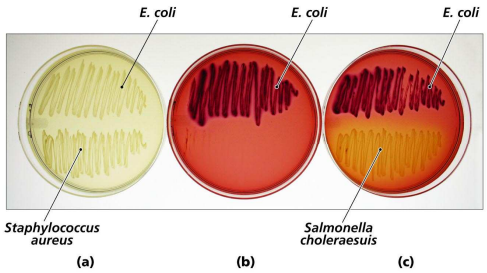
- Differential medium – allow differentiation of microbes in a microbial community. e.g. MacConkey agar differentiates lactose-fermenting from non-fermenting Gram-ve bacteria; thioglycollate broth differentiates microbes with different $O_2$ requirements.

## The use of blood agar as a differential medium



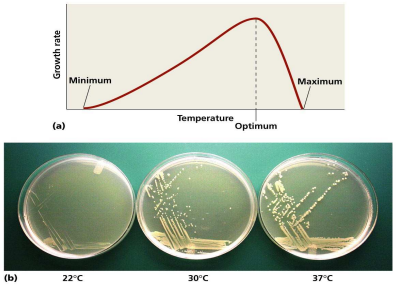



## MacConkey agar can be used as both a selective and differential medium



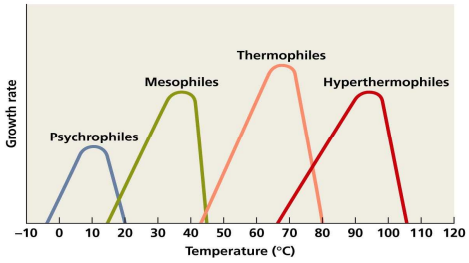
## Physical Requirements for Growth

- **Temperature**
  - Each microbe finds its niche in the environment
  - Range of temperatures for microbial growth
  - Minimum, optimum and maximum temperature





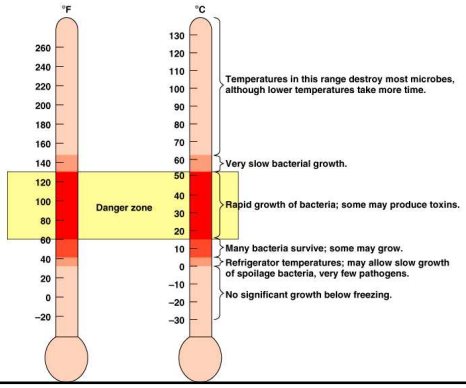

## Temperature requirement for growth






- **Psychrophile** – optimum below 15ºC
- **Psychrotrophs** – optimum between 20ºC-30ºC
- **Mesophile** – optimum between 25ºC – 40ºC
- **Thermophile** – optimum higher than 45ºC
- **Extreme thermophiles** – optimum above 65ºC

## Temperature



## Temperature

**Preserving Bacteria Cultures**:

In the presence of a protective agent (cryoprotectant), freezing stops all microbial activity without killing the bacteria. They can be recovered after long period of time.

- Deep-freezing:
  - 20% glycerol
  - -50°to -95°C
- Lyophilization (freeze-drying):
  - 20% skim milk, 12% sucrose 10% serum
  - Frozen (-54° to -72°C) and water is removed in a va cuum (ice to vapor)
  - Lyophilized bacteria are stored under vacuum and <8ºC

## The Requirements for Growth: Physical Requirements

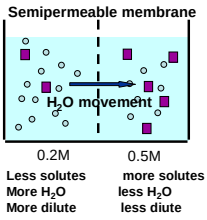
- **pH – acidity or alkalinity of a solution**
- **Effects of pH**
  - **Acidophile** – prefer below 7
  - **Neutrophile** – prefer 7
  - **Alkalinophile** – prefer above 7
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6

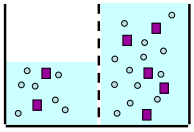
## The Requirements for Growth: Physical Requirements

- **Physical effects of water:**
  - **Osmosis - diffusion of water molecules across a selectively permeable membrane (e.g. cell membrane of a bacteria) with an attempt to equalize the concentration on both sides**
  - **A salt solution contain NaCl dissolved in water**
  - **solute = NaCl**
  - **solvent = water**
  - **Water move from a low solute concentration to a high solute concentration**
  - **Osmotic pressure – force exerted on a membrane by the solutions on both sides**

## Osmosis

- water flows from the solution with the lower solute concentration into the solution with higher solute concentration
- Equilibrium is reached once sufficient water has moved to equalize the solute concentration on both sides of the membrane, and at that point, net flow of water ceases (water molecules still move between two sides of the membrane)

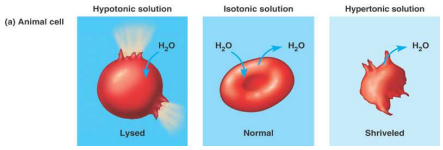



## Physical effects of water in the environments

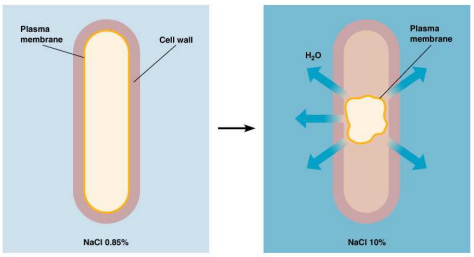
- **Isotonic**
  - External concentration of solutes is equal to cell's internal environment
  - Diffusion of water equal in both directions
  - No net change in cell volume
- **Hypotonic**
  - External concentration of solutes is lower than cell's internal environment
  - Cells swell and burst
- **Hypertonic**
  - Environment has higher solute concentration than cell's internal environment
  - Cells shrivel (crenate)

## Osmoregulation in animal cells




- **Burst (*lyse*) in Hypotonic solutions because water rushes into the cell**
- **Remain normal in Isotonic solutions**
- **Shrink (*shrivel*) in Hypertonic solutions because water flows out**

## Osmoregulation in bacteria




ISOTONIC HYPERTONIC

**What happen when you place bacteria in a hypotonic environment?**

## The Requirements for Growth: Physical Requirements

- **Osmotic Pressure**
  - Most microbes prefer isotonic solutions
  - Hypertonic environments (increase salt or sugar) usually cause **plasmolysis**
  - **Extreme or obligate halophiles** require high osmotic pressure e.g. halophilic archaebacteria
  - **Facultative halophiles** tolerate high osmotic pressure e.g. some *Stahphyloccocus* species

## The Requirements for Growth: Physical Requirements

- **Gas requirements** (Oxygen and $CO_2$)
  - **Aerobes** - use oxygen and can detoxify it
  - **Anaerobes**- can neither use or detoxify it
  - **Facultative anaerobes-** do not require oxygen but can use and detoxify it by enzymes
  - **Microaerophile** – requires a small amount of oxygen for growth
  - **Capnophile** – require higher $CO_2$ tension (3-10%) than normally found in the atmosphere

## The Requirements for Growth: Physical Requirements

- **Oxygen ($O_2$)**