

Metabolism

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Metabolism – sum of all chemical processes

– **Anabolism** – reactions that require energy to synthesis complex molecules from simpler one.

- Essential for growth, reproduction & repair

– **Catabolism** – reactions that release energy by breaking complex molecules into simpler ones

- Essential for providing energy for life processes; movement, transport & synthesis of complex molecules

Energy metabolism

- Catabolic reactions involve electron transfer – allows energy to be captured in high energy compounds such as ATP

- Electron transport related directly to oxidation & reduction

- enzymes are specific for a given substrate

- energy for reactions is stored as **ATP (adenosine triphosphate)**

- when a bond is broken energy released is used to run other reactions

- ATP is generated in some reactions and used to drive others

– **Oxidation** = loss or removal of electrons

- Many substances combine with O and transfer electrons to O₂; O₂ need not be present if there is another electron acceptor available

– **Reduction** = gain of electrons

- Energy is released and another substance must gain the electrons or be reduced at the same time

- Eg $2 \text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$: hydrogen is electron donor

(reducing agent) and oxygen is electron acceptor (oxidising agent)

- As oxidation & reduction must occur simultaneously the reactions in which they occur are called **redox reactions**

Respiration – 3 phases (Net production of ~38 ATP molecules)

1. Glycolysis – breakdown of glucose – ATP produced

2. Krebs Cycle – further breakdown, more ATP produced

3. Electron Transport Chain – electrons transported through a series of carriers, ATP produced

Fermentation

- no Krebs Cycle - no Electron Transport Chain

- only Glycolysis - net production of ~ 4 ATP

1. Ethanolic Fermentation

- yeast used to make beer, wine, by-product is ethanol

- yeast used to make bread – by-product carbon dioxide used as rising agent, ethanol is released during baking

2. Lactic Acid Fermentation

- make cheese, yogurt - e.g. bacterium *Lactobacillus*

Food Catabolism

1. **Carbohydrates** – sugar building blocks (e.g. glucose) enter via glycolysis

2. **Lipids** – broken down into Fatty Acids and Glycerol by enzymes called **Lipases**,

then glycerol enters via glycolysis and fatty acids via Krebs Cycle

3. **Proteins** – broken down into amino acids by enzymes called **Proteases** and join either glycolysis or Krebs Cycle

Anabolism – many components of glycolysis and Krebs Cycle are the starting point to make amino acids, fatty acids, nucleotides (all building blocks).

Energy metabolism in microbes

- Microorganisms versatile in ways they obtain energy

- Classified into groups on the basis of how they capture energy and carbon

- Carbon

 - Autotrophs (self feeding) – use CO₂

 - Heterotrophs (other feeding) – use organic carbon

- Energy

 - Light (photo-) Organic compounds (chemo-)

 - Chemoautotrophic

 - (chemolithotrophic) metabolism

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- **Sodium** – not required by all organisms; need often reflects the natural habitat of the organism

 - marine organisms require sodium, fresh water organisms do not.

Iron

- Iron – key role in cellular respiration – key component in cytochromes and iron-sulphur proteins involved in electron transport
- Under anoxic (anaerobic) conditions iron is in Fe²⁺ state & soluble; but under oxic (aerobic) conditions it is often Fe³⁺ & forms various insoluble minerals
- Bacteria have developed iron-binding proteins (**siderophores**) that solubilise such iron & transport it into the cell eg hydroxamic acid derivatives
 - Chelate Fe³⁺ very strongly – complex carried into cell – iron is split off and hydroxamate exits the cell and repeats the process
 - examples of siderophores: **Enterobactins** – *Escherichia coli* and *Salmonella Typhi murium*

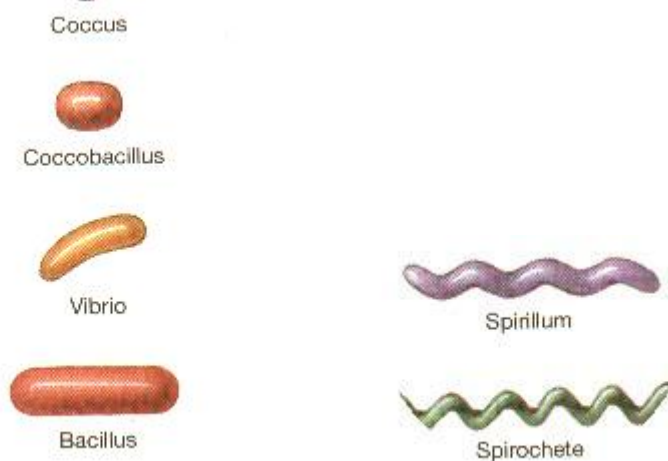
Micronutrients (trace elements)

- Critical to cell function even if only required in small amounts
- Micronutrients are metals – structural role in many enzymes
- Examples include cobalt, manganese, molybdenum nickel selenium zinc molybdenum, nickel, selenium, Growth factors
- Organic compounds – required in very small amounts and only by some cells
- Include vitamins, amino acids, purines, pyrimidines
- Most micro-organisms can synthesis all of these compounds – some require one or more to be pre-formed in culture environment
- **Vitamins** are the most commonly needed growth factors; most function as parts of co-enzymes
- Most commonly required vitamins are thiamine (vitamin B1), biotin, pyridoxine (vitamin B6) and cobalamine (vitamin B12)

Categories of growth media

- **Enriched**
 - Nutrient medium containing enrichments such as blood or serum or yeast extract – Enrichment provides additional growth factors for more fastidious organisms (includes many pathogens – eg Blood agar
 - Enrichment – Contains special nutrients that allow the growth of a particular organism that may be present in low numbers and so masked by other organisms

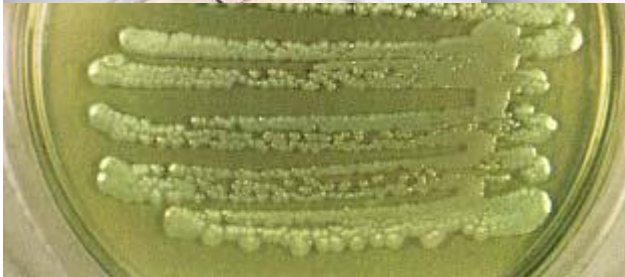
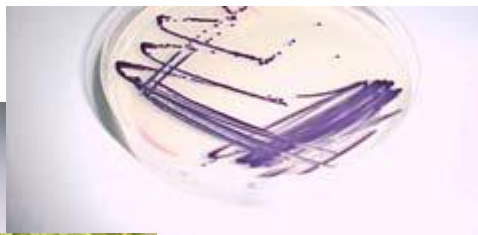
- Usually a broth culture medium
- Eg Rappaport's medium for salmonella, Enterococcosal Broth for enterococci
- **Selective**
 - Encourages the growth of some organisms but suppresses the growth of others
 - Eg Mannitol salt agar for isolation of *Staphylococcus aureus*
- **Differential**
 - Contains a constituent that causes an observable change (change in color or change in pH) in the medium when a particular biochemical reaction occurs
 - eg fermentation of lactose in MacConkey medium causes a pH change – lactose fermenting colonies pink, non-lactose-fermenting colonies colorless
- Combined selective and differential media
 - Eg MacConkey Agar – contains crystal violet & bile salts which inhibit Gram-positive bacteria plus lactose and pH indicator (see previously Categories of growth media salt agar for isolation of *Staphylococcus aureus*)
- Stain the organisms so they are visible under the microscope



- (1000X magnification) • Gram stain is a common staining reaction used
- Morphology of organisms determined (rod, coccus etc)
 - Culture of organisms on agar plates

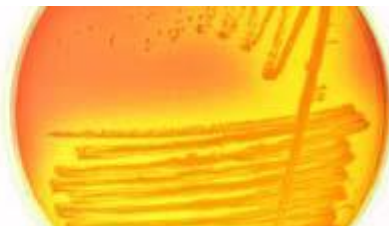


Colony morphology



Pigment production

Differential media



Mannitol salt Aga

Differential & selective media • *Salmonella typhimurium* on MacConkey Agar: growth, colorless colonies
• *Escherichia coli* on MacConkey Agar: growth, with pink colonies

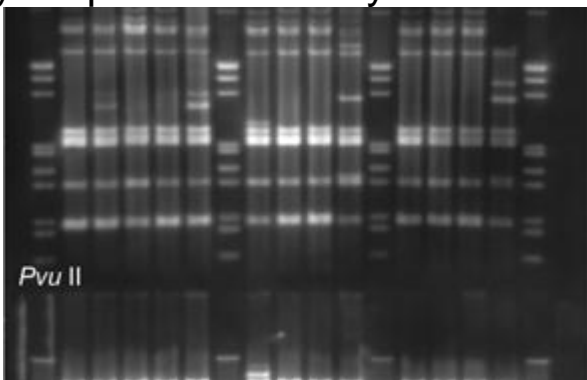


MacConkey agar



Metabolic characteristics

- Fermentation of sugars/carbohydrates
 - Break down of polysaccharides (starch) and proteins (casein)
 - Break down of amino acids (tryptophan
 - indole)
 - Utilization of different carbon and nitrogen Compounds
 - Requirements for enriched media (eg
• blood agar, chocolate blood agar ..)
- Molecular techniques:**
- Rib typing
 - Retracement Fragment Length Polymorphism
 - DNA hybridization
 - rRNA sequencing



Clinical & diagnostic methods for isolation & identification of bacteria

