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 H2 + O2 → 2H2O: hydrogen is electron donor (reducing agent) and oxygen in 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
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

- **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: • *Escherichia coli* on MacConkey Agar: growth, colorless colonies 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 (e.g., 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