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

- 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

• **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







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 (tryptopan
- □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