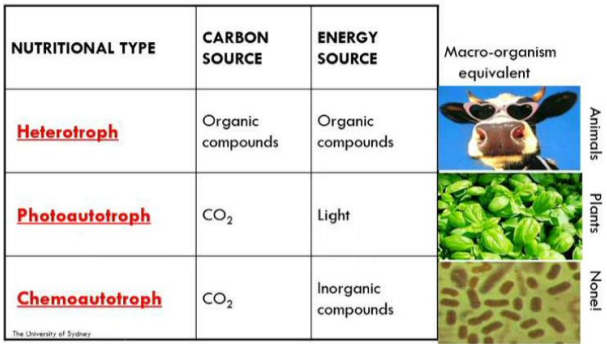
PHAR2812: Microbiology and Infection

Micro Intro & Techniques

- microbiology very important: many microbes useful, not inherently harmful to us

Microbes can…	Notes/Examples	Relevance to pharmacy
Cause disease	• Bacteria – tuberculosis • Fungi – tinea • Protozoa – malaria • Viruses – HIV	• Develop and formulate vaccines/antimicrobial drugs • Pharmacists must practice safely
Destroy pharmaceutical products	• Cause infection • Spoilage and degradation • Change product features; e.g. colour, taste, odour	• Produce contaminant-free products • Inhibit microbial growth in products
Help manufacture pharmaceutical products	• Antibiotics; e.g. penicillin • Vitamins and hormones	• Produce drugs using microbes

- no macro analogue for chemoautotroph
- media used for growing microbes:
  - broth: contains nutrients required for growth, shaken to mix cells, nutrients and oxygen, homogeneous and useful for growing large numbers of microbes → cotton wool stopper allows entry of air but not microbes
  - agar plates: also contain nutrients, solidified with agar, heterogeneous, useful for growing small numbers of cells → loose lid allows air but not microbes to enter



- complex vs. defined media: ingredients derived from organisms (exact composition unknown) or ingredients are pure compounds
  - can predict growth in defined media
- general purpose, selective or differential media
  - general purpose allows growth of many microbial types, e.g. broth
  - selective favours growth of one microbial type, e.g. media containing antibiotics
  - differential gives different colour or visual reactions, e.g. pH indicator
- pure vs. mixed cultures: need pure for research (unnatural as natural microbial communities are mixed)
  - many microbial types can't be isolated in pure culture; pure culture not representative of microbial diversity or microbial interactions
  - pure cultures will behave differently to microbial mixtures
- study of individual cells needs microscopy
  - resolution = smallest distance between two points identified as separate
- fixing and staining critical to view cells: fixing immobilises to help visualisation, staining provides contrast
- Gram staining: most widely used stain for bacteria

- G- = thin cell walls do not retain dye and it is easily washed away (pink)
- G+ = thick cell walls retain crystal violet dye after alcohol rinse (purple)
  1. crystal violet – primary stain
  2. iodine – mordant (helps dye to stick)
  3. acetone:alcohol – decolouriser (shrinkage aids dye retention)
  4. safranin – counter-stain

- phase-contrast microscopy allows one to see live and unfixed samples
  - can determine motility, natural morphology, some internal structures without staining
- electron microscopy is a solution for seeing viruses etc. which are smaller than visible light wavelengths (and thus cannot be seen with high resolution under a light microscope)
  - scanning electron microscope (SEM): electrons bounce off sample surface
  - transmission electron microscope (TEM): electrons sent through a thin section of sample
  - very labour-intensive preparation process, takes a lot of time

Major Infectious Diseases (HIV, malaria, TB and influenza)

- viruses have simple structures: simple, small genome and envelope, dependent on host for replication
- HIV:
  - genome = RNA virus (retrovirus)
  - reverse transcriptase enzyme
  - targets human immune cells (T-cells)
  - transmission via blood, saliva, semen and vaginal secretions
- HIV previously known as Acquired Immune Deficiency Syndrome (AIDS), before being shown it was due to a virus
  - HIV is the virus; AIDS is the clinical phase during which symptoms start appearing
- virus particle of HIV has a protein in virus membrane; this protein binds to a receptor protein CD4 on host membrane (T-cells)
- reverse transcriptase uses ssRNA molecules as template for first DNA strand; using this DNA strand as a template, dsDNA is made

- as soon as dsDNA generated, can incorporate itself into genome of T-cell; as long as T-cell is alive, can replicate with its host
  - poses difficulty in treatment; if targeting HIV infected cell death, effectively killing all immune cells
- HIV occurs as HIV-1 and -2; both are related to Simian Immunodeficiency Virus (SIV) which is found in other primates and does not seem to have symptoms in original monkey hosts
- first indications of HIV: USA 1981, clustered cases in homosexual men + cases of Kaposi's sarcoma
  - distribution suggested infectiveness; then found in IV drug users and became best hypothesis (now backed up by evidence)