General Microbiology – Full course notes

Lecture 1: Historical and Current Aspects of Microbiology

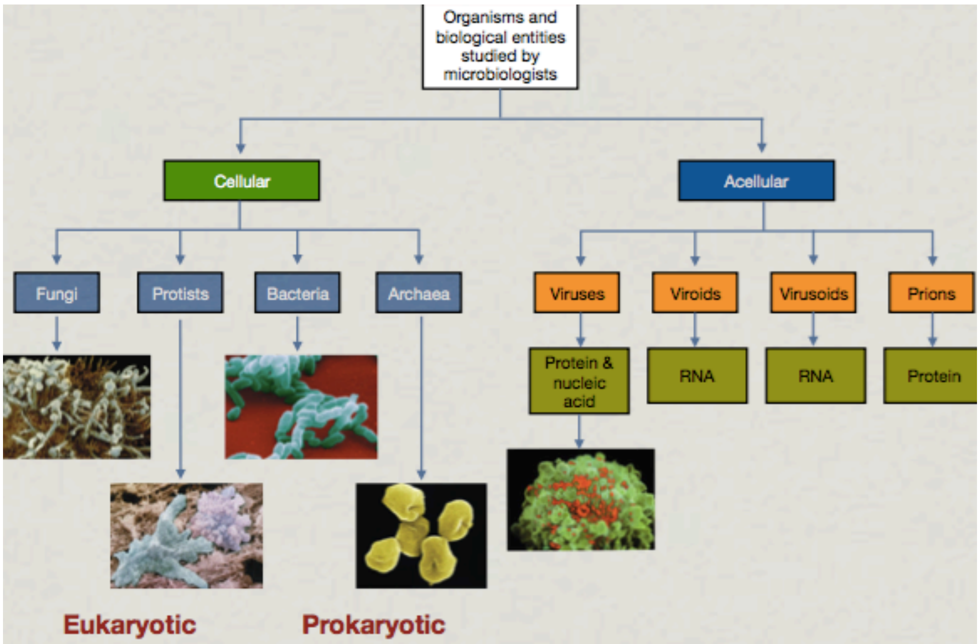
Learning Objectives
Be able:
- To define **Microbiology**
- To **differentiate the major characteristics** for each type of microorganism in the microbial world
- To **identify the contributions to microbiology** & **germ theory** made by past microbiologists
- To appreciate the **genomic revolution** occurring in Microbiology

What is Microbiology?

- Microbiology is the study of organisms too small to be seen with the naked eye
- **Eukaryotes:** have a membrane bound nucleus, more complex and larger than pro
- **Prokaryotes**: Lack a membrane bound nucleus
- Single cells that can grow independently and do not rely on other cells to live
- Might exist in a multi-cellular community e.g. grime in toilet

The Microbial World

Cellular – can replicate on their own, acellular cannot.



History of Microbiology

- Stelluti, Hooke, Van Leeuwenhoek, *first people to observe and describe microorganisms*
- *Spontaneous Generation:* Living organisms could develop from non-living matter
- *Louis Pasteur:* disproving the theory of spontaneous generation

Germ Theory

- Infectious disease was thought to be caused by supernatural forces
- Support for germ theory came from a number of studies
    - Agostini Bassii (1773-1856) – fungi on silk worms
    - M.J Berkeley (1803-1889) – potato blight caused by fungi
    - Joseph Lister (1827-1912) – sterilizing equipment reduced infection

Koch's Postulates

- Suspected pathogen must be present in all cases of disease and absent in healthy
- Pathogen must be grown in pure culture
- Cells from pure culture must cause disease in healthy animal
- Suspected pathogen must be reinsulated and shown to be same as the original (rod and rod etc.)

N.B. there are some cases where we cannot use Koch's postulates

Size

DNA/RNA < VIRUSES < BACTERIAS < EUKARYOTIC CELLS < SMALL ORGANISMS

Microbiology Tools

- Light Microscope can allow you to see microscopic but not small as viruses
- Culturing: agar plates, liquid mediums etc.
- DNA sequencing: sequencing genes, genomes, comparing, identifying virulence or other genes. Pure culture → Genome → Ask questions. Meta-genome = from a number of cells e.g. soil, sample from gut etc. allows to see what genes define this environment

Microbial Diversity

Learning objectives:
- To recognize microbial diversity at multiple levels
- To identify different ways for describing diversity in microbes

Diversity of Microorganisms

Diversity: The range of features of degree of difference between organisms in a particular environment.
Three types of microbial diversity:
1. Physical/structural e.g. cell shapes, gram stain
2. Biochemical/metabolic e.g. energy sources, secondary metabolites
3. Genome i.e. DNA sequence

1. Diversity of Morphology – Prokaryotes

Cocci (Spheres) – 1 um diameter
Arrangements:
- Diplococci: pairs
- Chains (e.g. streptococci)
- Clusters (e.g. Staphylococci)
- Tetrads (4 cocci in a square)
- Sarcinia (8 cocci in a cube)

Bacilli – Rods – 1um x 3um

- Coccobacilli (very short rods)
- Filamentous (long rods)
- Vibrios (curved rods)
- Blunt or Square ends

Arrangements: cells arranged singly, in short chains, palisade or Chinese Lettering

Less Common:
- Spirilla - rigid helices
- Spirochetes - flexible helices (usually quite long, internalized flagella)
- Coryneform - variable appearance (usually rod-like)
- Pleomorphic - variable, irregular shapes, occasionally branched
- Flattened rectangles, triangles & trapezoids (rare)

1. Diversity of Morphology – Eukaryotes

- Morphologically more complex than prokaryotes
- Protists (diverse taxa)
- Fungi (one taxon)

1. Diversity of Morphology – Viruses

- Morphologically complex, despite being acellular

2. Diversity of Metabolism

- Metabolism refers to all the chemical reactions in a cell or biological systems
- Microorganisms show enormous metabolic diversity
- Due to highly variable environments, microbes have evolved diverse ways of obtaining energy and matter for growth

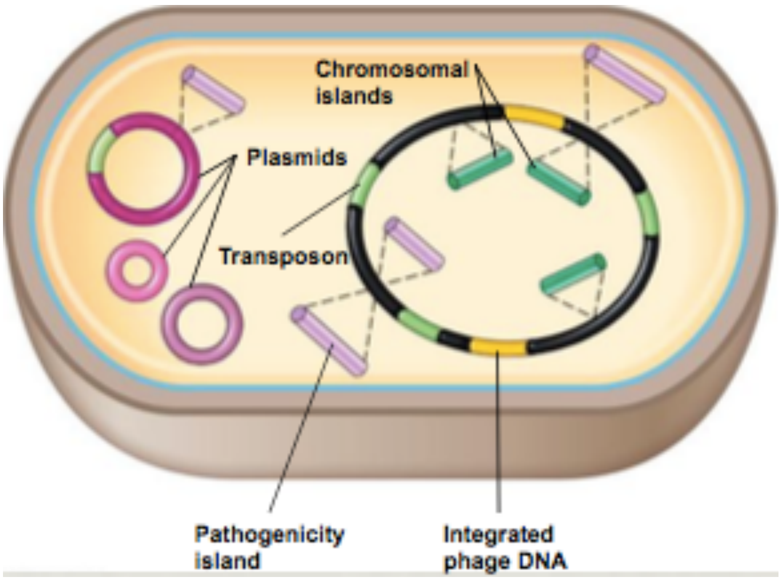
3. Diversity of Microbial Genomes

- **Genome/Genotype:** the genetic makeup of a cell
- **Core Genome**: The complement of genes that make up a species
    - Genes (DNA) encode all the information needed for cell structure and metabolism
    - Genes reflect all aspects of physical and chemical diversity
    - The DNA sequence of genes and genomes displays great diversity amongst microorganism

Exit Questions:

Diversity of genome and meta-genome:

- *Pure culture gives a genome whereas an environment gives us a meta-genome, tells us what are the vital components of the environment*
- *Genome – all the DNA in a clonal cell line (strain)*
- *Genome diversity – mutation and lateral gene transfer*
- ***Core genome –** common genes between strains in species (black in figure)*
- ***Pan Genome –** different genes between strains in species (Colours in figure 1)*



LECTURE 2: *Microbial nutrition*

Learning objectives:

- *Recall the nutrient classes required for microbial growth*
- *Name a use for each of the 4 macronutrients (**carbon, nitrogen, sulfur and phosphorus**) needed in large amounts for growth*
- *Name a use for cofactor macronutrients, trace elements and growth factors*
- *Classify microbes into nutritional types based on their requirement for energy and carbon*

1. *Essential macronutrients*

Composition of microbial cells (composing more than 95% dry weight:

- Essential macronutrients (C, H, O, N, S and P)
- Required in large amounts: components of carbs, proteins, lipids and nucleic acids

Nitrogen, Phosphorus and Sulfur:

- All organic matter contain C, H & O but also N, P & S
- These can be from the same supply source as carbon or from **inorganic** sources

Nitrogen

- Needed for synthesis of amino acids , nucleotides, some carbs and lips
- As enzyme cofactors
- Supplied in many ways:
- Some m.o can use nitrogen from amino acids and organic molecules
- Some incorporate ammonia directly and some reduce nitrite to ammonia
- Some bacteria "fix nitrogen" (collect atmospheric nitrogen & reduce to NH_4^+)

Phosphorus

- Needed for nucleotides (including ATP), phospholipids, cofactors and some proteins and cell components
- **All m.o. Use inorganic phosphorus**
- Most incorporate p directly
- Low phosphorus can limit growth as so important in ATP production

Sulfur

- Needed for syntheses of amino acids **cysteine and methionine,** some carbs, biotin and thiamine
- Usually supplied as sulfate or via organic sulfur compounds

2. Cofactors - macronutrients

Potassium (K) and Magnesium (Mg), (Calcium (Ca) and Sodium (Na) on occasion) e.g. for marine animals as sodium is an important requirement.

Cofactors for enzymes, (enzymes will not work if these cofactors aren't present in quite high levels), complexes with ATP, stabilise ribosomes and components of cytochrome and electron-carrying proteins.

- Cations and enzyme activity
- Stabilisation of membrane and DNA + function of ribosomes (Mg & K)
- Structure and function of ribosomes (Mg & K)

3. Trace Elements

Micronutrients or trace elements includes: Iron (Fe), Manganese (Mn), Zinc (Zn), Cobalt (Co), Molybdenum (Mo), Nickel (Ni) & Copper (Cu).

- Required in trace amounts for certain enzymatic functions or protein stabilisation
- In culture, these are provided in water or media components or contaminants (water on glassware etc.)
- In nature, these are abundant and rarely limit growth
- **E.g. zinc finger proteins - DNA binding – zinc allows dna to take up its binding structure**
- Cations and cytochromes (e- transfer), Fe & heme, fe and iron sulfur proteins

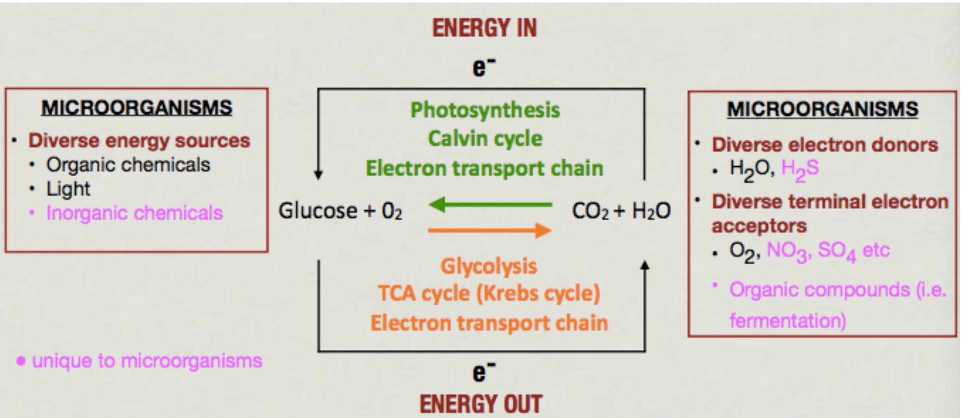
4. Growth Factors

Some microbes are unable to synthesis their certain molecules and these must be obtained from the environment or provided in growth medium. Growth factors are specific to certain organisms
E.g. Lactobacillus - lost ability to make its own amino acids, therefore to grow you have to add them yourself.
Three categories:

- Amino Acids (synthesis of proteins) e.g. *Lactobacillus* spp. And requirement for amino acids
- Purines and pyrimidine's (synthesis of DNA & RNA)
- Vitamins (co-enzymes and functional groups of certain enzymes)
- **Microorganisms also have specific requirements that reflect their specific morphology and environment.** E.g. diatoms need sialic acid to construct their cell walls of silica.

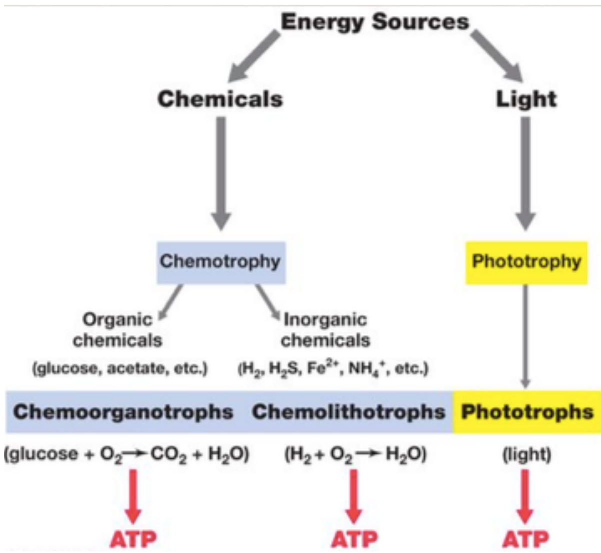
Metabolic Diversity:

All life is an oxidation/reduction reaction:
ΔG0'=Gf0[C+D] - Gf0[A+B]



- Electron transfer to get energy out in the form of ATP

- Reverse is photosynthesis, use energy of light to make molecules
- Microbial cells can also use organic compound as energy sources e.g. fermentation.
- Can take in inorganic sources as energy source and use it to grow
- This is why they are really important in nutrient cycling in the environments



<u>Nutritional Types</u>

All organisms require energy and a carbon source.

Chemotrophic – energy from chemicals

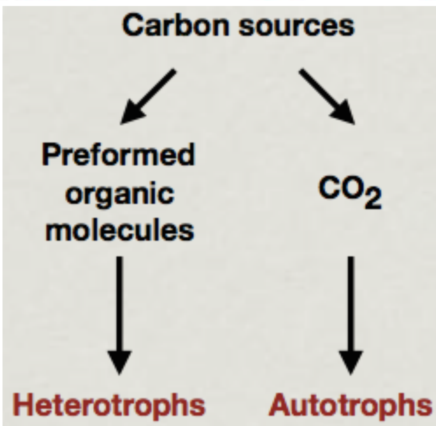
Chemoorgantophoy – energy from organic chemical

Chemolithotrophy – energy from inorganic molecules

Example: Photoautotrophs

Bloom of cyanobacteria

Energy: Light



Carbon: CO_2

Splits H_2O for electrons win photosynthesis producing oxygen

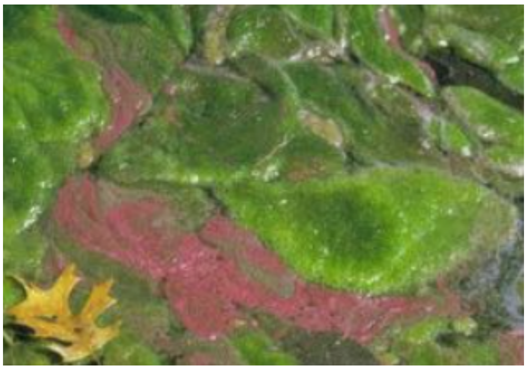
Purple sulphur bacteria

Energy: Light

Carbon: CO_2

Splits H_2S for source of electrons in photosynthesis producing sulphur

E.g. Chemolithotrophs (rock eaters)



- Nitrobacteria winogradsky
- Energy = inorganic chemical NO

<u>Microbial Culture Media</u>

Learning objectives:

Be able to:

- *Differentiate between defined and complex media*
- *Construct a medium using your knowledge of what microbes need for growth*
- *Define supportive, enriched, differential and selective medium*

Culture Media

Must contain all he nutrients required by the organism to grow:

Classified based on:

1. Chemical constituents (defined or complex)
2. Physical Nature (liquid, semi-solid or solid)
3. Function (supportive, selective or differential)

1. Chemical constituents

***Defined**: All components and constituents (and concentrations) are known*

***Complex**: Contain some ingredients that are of unknown composition or concentration*

Culture media – Defined: E.g. A chemically defined medium for growing a typical chemoheterotroph, such as *Escherichia coli.* All medium is defined and used in growth, macronutrients C, H, O, N, S, P and cofactor macronutrients: K & Mg.

Example of culture media: complex:

- Comp of nutrient agar, a complex medium for the growth of heterotrophic bacteria
- **Peptone** (protein hydrolysates prepared by partial digestion of various protein sources)
- **Extracts** (usually yeast or beef extracts)
- Provide carbon, nitrogen, sulfur, vitamins and minerals in the form of organic material such as amino acids, nucleotides, peptides, organic acids etc.

Culture Media – Physical Nature

- Agar (sulfated polysaccharides used to solidify liquid media such as L-broth

- Extracted from red algae
- Melts at 90C but once melted doesn't harden until 45 C so can be cooled to a temp for handling and addition of heat sensitive compounds (e.g. vitamins or antibiotics)

2. Culture Media – Function

- **Supportive**: supports many organisms e.g. tryptone soy agar

- **Enriched**: general purpose media enriched with blood or other special nutrients to support the growth of fastidious bacteria e.g. blood agar

- **Selective**: allows growth for particular microorganism while inhibiting the growth of others e.g. MacConkey agar – selects for enterics (i.e. bile salts)

- **Differential**: Distinguished on different groups of microorganisms based on their biological characteristics. E.g. blood agar hemolytic vs. non hemolytic & MacConkey Agar – lactose fermenter (red) versus non-fermenter (pale)

Exit questions

Questions From Last Weeks Exit Ticket

- ***Are there any chemotrophs that are autotrophs, or phototrophs that are heterotrophs?***
- ***Can an organism be a heterotroph and an autotroph?***

Short answer, Yes. ***Mixotrophy.*** *Depends on the environmental conditions.*

Gram positive and gram negative features:
Gram-positive thick cell wall, glycine interbridge, telcholc acids, no LPS and cytoplasmic membranes
Gram negative thin cell wall, direct link, no telcholc acids, LPS, 2 membranes cytoplasmic + outer