Session 1

Fundamentals of Microbiology
Session overview

• Classification
• Microbial nomenclature
• Microbial growth
• Microbial death
• Spore formation
Classification

The Five Kingdom system is used to classify all organisms.
Differentiation between organisms is based upon
• cellular structure (morphology)
• metabolism (biochemistry)
• reproduction
• DNA

Five basic types of micro-organism:
algae, protozoa, fungi, bacteria and viruses

The first four fall within three of the kingdoms:
Fungi, Protista and Monera

Viruses do not fall within the Five Kingdom scheme
**Gram reaction (Gram positive and Gram negative)**

- Developed in 1884 by Danish physician Christian Gram
- Only used for bacteria
- It is the first step in identifying unknown bacteria
- It is based upon a differential stain—**Gram positive** cells retain the stain and **Gram negative** do not
- The difference is due to differences in cell wall composition
Microbial nomenclature

• All organisms have two names-the first signifies the *genus* and the second signifies the *species*

• This is a binomial system-devised by Linnaeus

• Genus name can be abbreviated, species name should not be

e.g. *Campylobacter jejuni* can be abbreviated to *C. jejuni*

    not *Campylobacter j.*
Microbial growth

Growth is the process by which:

- individual cells increase in size and mass
- the population increases in number

Basically *metabolism* and *reproduction*

Reproduction for bacteria and fungi tends to be asexual-*budding* or *fission*
• Growth on solids is in the form of **colonies**-visible to the naked eye

• Within solid foods **micro-colonies** will appear

• In liquids, growth appears as increasing **turbidity**

• Moulds grow as a filament where cells stay attached (described as **hyphae**), but not all the cells will grow—only those at the tip of the filament
Two types of growth system—**open** and **closed**

- Within an **open system**, nutrients are constantly replenished and population growth will not stop. For example, biofilms in the pipework of food factories will have constant access to fresh nutrients.

- Within a **closed system**, nutrients and other factors will become limited and growth will eventually cease. For example, in a bottle of milk the nutrients will run out.
There are four distinct growth phases for a unicellular organism in a closed, liquid culture:

1. **Lag Phase**: Initial period of adaptation.
2. **Log Phase**: Period of rapid exponential growth.
3. **Stationary Phase**: Period of stable growth rate.
4. **Death Phase**: Period of decline in cell numbers due to resource depletion or accumulation of metabolic byproducts.
Growth phases

• Lag phase
  No growth occurs because the cells are adapting to the new environment

  Length of period varies, dependent on culture conditions

• Log phase
  Cells are growing exponentially or logarithmically—the population doubles within a certain time—depends on exact conditions, but can be as little as 20 mins

  This is called the generation time
• **Stationary phase**
  A nutrient depletion or toxic by-product build up stops the cells from metabolising or reproducing—the cell numbers remain static

• **Death phase**
  In this phase the cells start to die and the viable population decreases

  Population death is also exponential—not all the cells die at once
Methods for measuring growth

- **Colony counts**
  - *aerobic colony count* for all viable bacteria
  - *selective counts* for particular organisms/species

- **Direct counts**
  - using a microscope-viability can be determined with special stains

- **Absorbance measurement**
  - for clear liquid cultures only
Microbial death

Death can be defined as the inability of a cell to both metabolise and reproduce due to irreversible cell damage.

Death of cells leads to loss of viability - normally defined as the ability to grow and reproduce.

i.e. producing visible colonies on a plate or producing turbidity in liquid.
However, lack of growth is not always the same as death.

Some species may enter a **viable but non-culturable phase**—cells do not reproduce in culture but may retain viability and if pathogenic, the ability to infect.

Examples are *Listeria* and *Campylobacter*.

This is possibly a stress reaction and may occur in aquatic environments.

Still just a theory!
Survivor curves

When the population (rather than the individual cell) is considered, microbial death can be given a mathematical basis and various parameters can be calculated.

D values give the time taken to reduce the population by one log (e.g. 1000 to 100).

What does this mean?

It basically gives a measure of the resistance to factors such as heat and radiation.
Survivor curve

- Time
- Log survivors
- One log cycle
- D value
Other more complex parameters can be calculated from D values e.g. Z and F values

**Why is this relevant?**

D and other values are the basis for all food processing operations-pasteurisation, sterilisation and canning

e.g. Cans undergo a 12D cook for *Clostridium botulinum*- in other words the cook is aimed at reducing *Cl botulinum* spores by 12 logs

**For example, this means a reduction from 10^6 to 10^{-6} spores per gram**
Spore formation

For moulds, spore formation is a method of reproduction.

Moulds will release millions of spores, which will then move through air or liquid, and if they land on a suitable surface they will germinate and produce vegetative mould cells.

It is solely a reproductive function.
Some bacteria also produce spores—however, this is a \textit{survival} mechanism.

Bacterial spores are known more correctly as \textit{endospores}, since they are produced within the cell and only released upon the death of the cell.

Endospores are dormant and will only germinate to produce a vegetative cell when conditions are suitable for bacterial growth.

Bacterial spores are very resistant to heat, alkali, acid, dryness and can survive for years.
Session summary

• Classification and nomenclature are important to ensure consistency of identity and naming

• Growth is an increase in cellular mass and population

• Unicellular growth in closed liquid systems occurs in four phases

• Death curves can be used to calculate appropriate heat treatment regimes for foodstuffs

• Bacterial endospore formation is an important survival mechanism