Topic 1: Scope of Food Microbiology.

Development of early food microbiology (<1900)

- Leeuwenhoek, 1676 – first observed bacteria
- Appert, 1804 – preservation/heat glass bottles, appertization
- Pasteur, 1870- pasteurization, pathogens vaccines etc.
- Koch, 1880s- agar plates, pathogens etc.
- Gram, 1884- developed gram stain

The aim of food microbiology is to ensure the supply of safe and wholesome food to the consumer.

Why study food poisoning and spoilage.

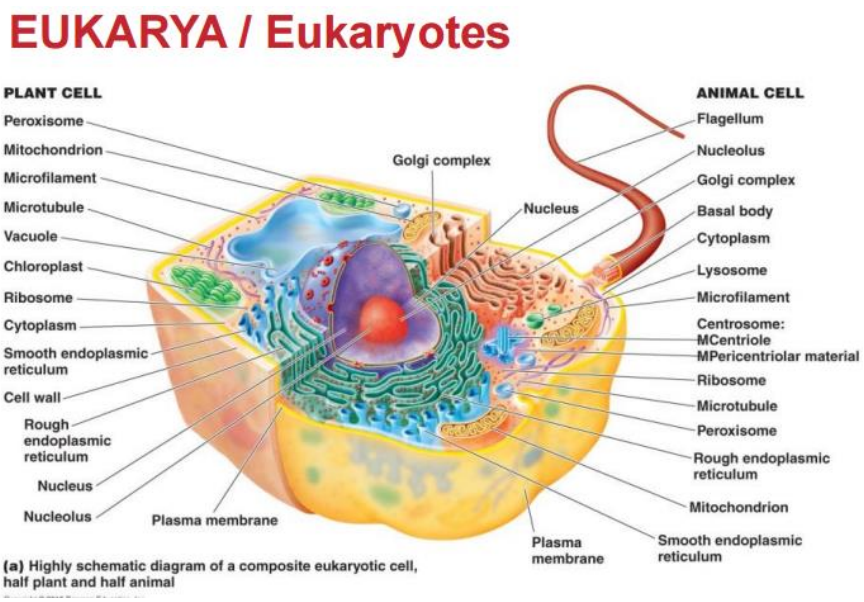
- Reduce disease: have an understanding of microbe-food interactions.
- Reduce food losses: improve food storage and preservation. 10-25% reduction

Consequences of undesirable microbes in food (most prevalent in developing countries).

- Disease outbreaks, illnesses (deaths) – 5.4m Australians contract disease = 120 deaths
- Food spoilage- ie. 10% global grain supplies lost after harvest through spoilage.
- Product recall and waste management
- Severe financial losses for a company = job losses= lawsuits (American peanut butter company) – loss of manufacturing licence, export embargos, political/international arguments etc. estimated 1.25 billion annual cost of food poisoning in Australia.

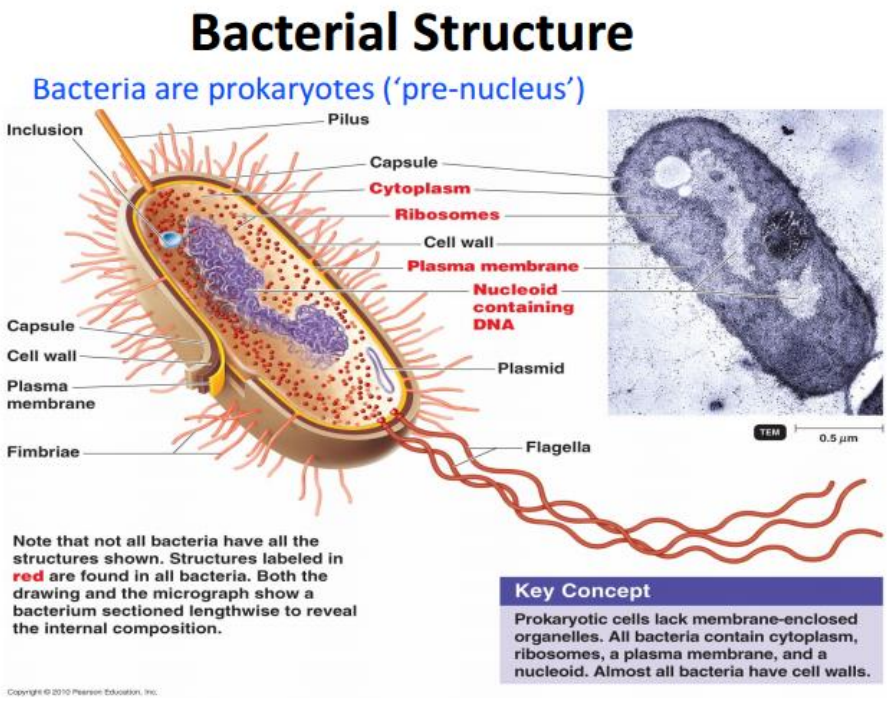
Micro-organisms

- Are common in the biosphere and play a primary role in nature
- Are opportunistic and take advantage of substrates.
- Self-perpetuate using chemical conversions (Binary fission) – fast (e.coli = 20mins) and exponentially in sequential waves.



(a) Highly schematic diagram of a composite eukaryotic cell, half plant and half animal

- To preserve foods and control spoilage, we limit the ability of micro-organisms to convert chemicals in food into by-products. Allow certain microorganisms to dominate.



Eukaryotes

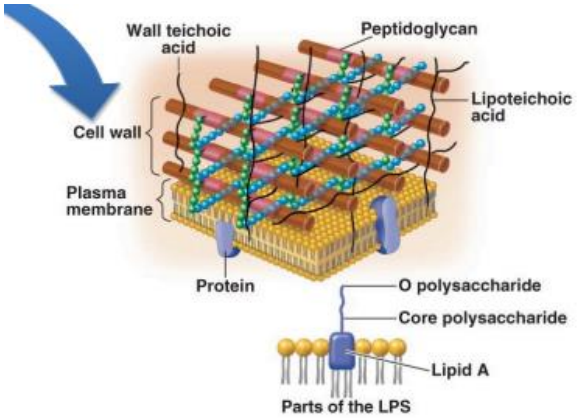
- ie. yeasts
- Have a singular whip like flagellum (singular, flagella= plural) for motility in animals

Bacteria are prokaryotes

- All contain cytoplasm, ribosomes (simpler and differ in sequence to those of eukarya), plasma membrane and a nucleoid containing DNA.
- Prokaryotic- therefore lack membrane- enclosed organelles (no NUCLEUS, mitochondria etc)
- Flagella: to move from undesirable locations ie. pH
- Fimbriae: to latch onto surfaces
- Usually a single, circular DNA chromosome
- Genome sits in the cytoplasm, so replication, transcription and translation all occur in cytoplasm.
- Plasmids: contains genetic DNA
- Enormous range of metabolic pathways, grow anaerobically, breakdown lactose, produce specific end products, resistant to salt etc.

Bacterial cell structure

- Capsule: glycan carbohydrate matrix, more resistant to drying and multiplying when moist
- Most bacteria are surrounded by a cell wall, shape is determined by peptidoglycan
- Osmotic stress- damage cell wall and become vulnerable.
- Reaction to gram stain shows the cell wall composition



Peptidoglycan

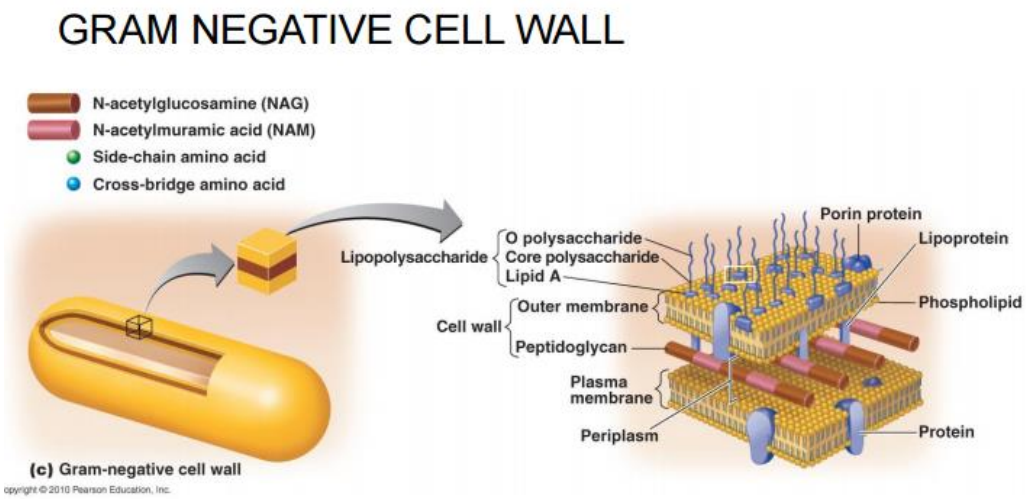
- Amino acid side chains differ in gram positive and negative cell
- B1,4 glycosidic bond susceptible to hydrolysis by lysozyme
- Syntehesis of (P) is disrupted by penicillin and synthetic derivatives.

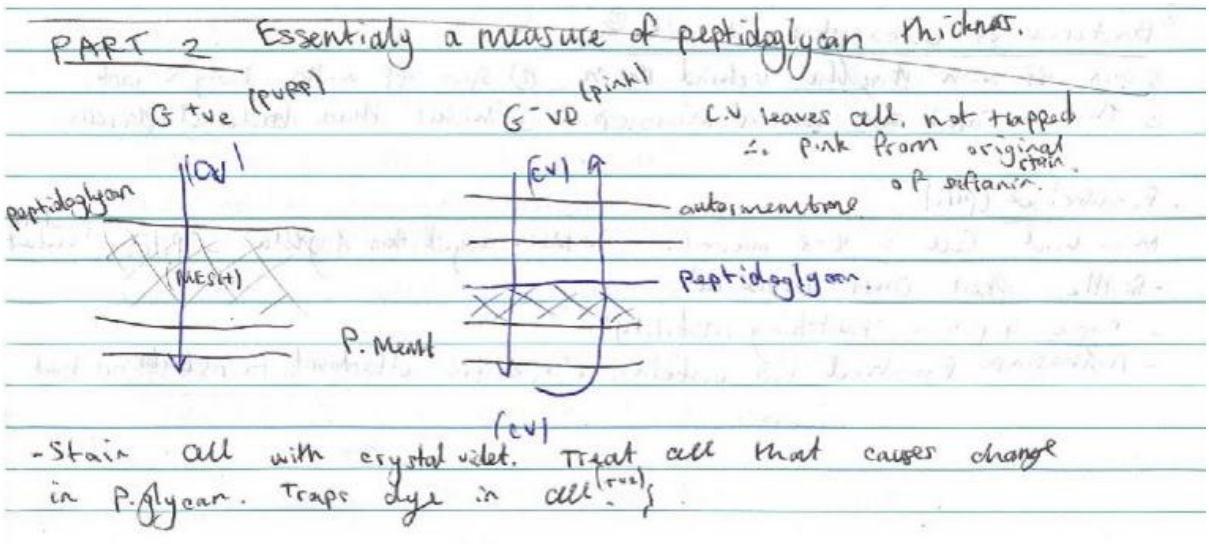
Gram positive cell wall (purple once stained)

- Hydrophobic tail, hydrophilic (filter) phosphate head – nutrient uptake, waste output
- Mesh= thick peptidoglycan (single layer), tethered to plasma membrane

Gram negative cell wall (pink once stained)

- Phospholipid bilayer with proteins embedded
- Thick peptidoglycan wall (rigid polymer of NAG, NAM) with outer membrane





Bacterial motility: Flagella: built from protein components which are strongly antigenic (varied)

- Long helical filaments that are used for propulsion
- Allow bacterial cells to display chemotactic and phototactic responses
- Peritichous: bacteria with flagella covering entire surface
- Polar: bacteria with flagella restricted to single pole of the cell
- Lophotrichous: bacteria with flagella in tufts on cell
- Too thin to see by light microscopy – 20nm diameter

Eukaryotic vs Prokaryotic flagellum

- Bacterial flagella spin off with flagella behind them and can be seen under microscope
- Eukaryotic flagella spin off with body at back, and seen under ‘scope (thicker)

Bacterial pili (fimbriae)

- Also surface projections but are more rigid than flagella and do not rotate
- Composed of pilin protein

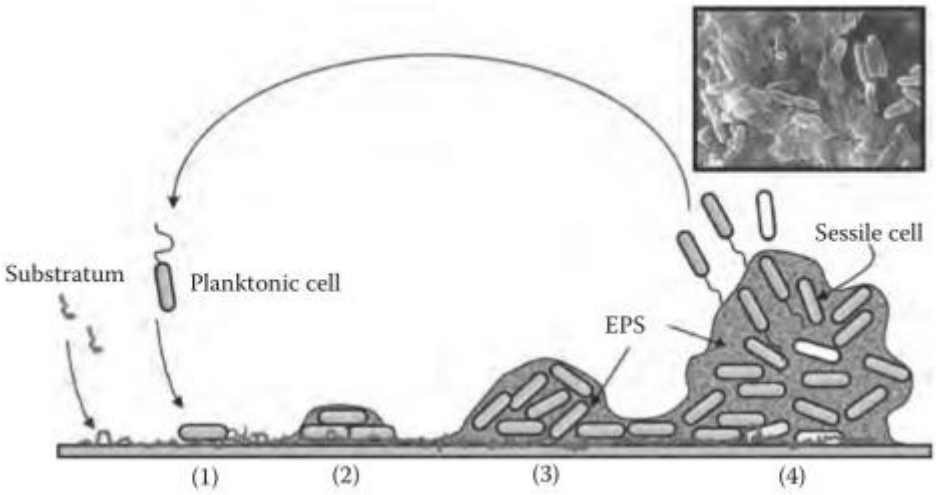
- Type IV pili are involved with twitch motility
- Used for a attachment to surfaces and cells
- Some have adhesins (tip proteins) that provide specific attachment molecules on the surface of host cells
- Cells with lots of pili may be resistant to phagocytosis

Microbial biofilms, and capsules

- Capsule is a slimy/gummy layer outside the cell- protection
- Capsules are composed of polysaccharide-protein complexes
- Most bacteria can form biofilms, multilayered 3D cell pop^{n} that stick to surfaces
- Form extracellular polymetric substance (EPS) to form protective gel around the cells, help stick to surfaces, resist action of external pH changes, disinfectants, desiccation etc
- Microbes normally try to attach to surfaces, taking up niche space preventing competitors, often attachment is due to thin capsules or EPS.
- Lifestyle advantages= resist removal, protection from drying, chemicals and antibiotics

Microbial biofilms

1. attachment to substratum 2. Microcolony: Dock to planktonic cell using flagellum to desirable surface 3. Maturation, produce EPS (gummy layer) 4. Detatachment/dispersion – sessle cell, antibiotic resistance, some cells return to planktonic

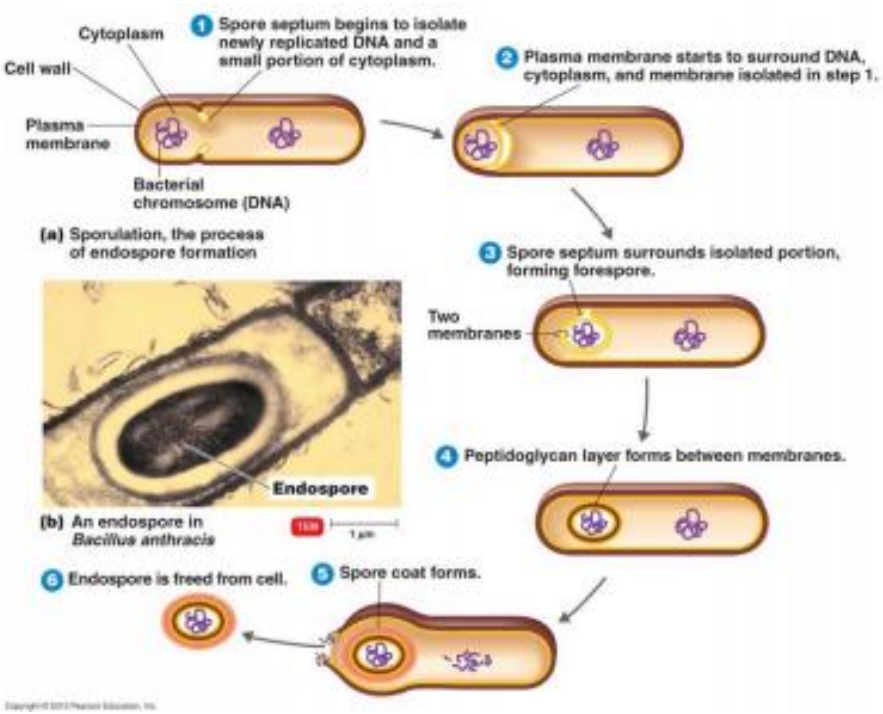


Endospores- matchstick appearance:resting structure formed inside some bacteria. HEAT RESISTANT

- Terminal spores haven't taken up gram-stain = resting form = resist drying, disinfect etc.
- Highly resistant resting bodies –UV, high temperature, desiccation, disinfectants, freezing /thawing (dormant), therefore resist change until desirable conditions then germinate.
- Far more resistant that vegetative forms, and can remain viable for many years.
- Clostridium and Bacillus species (food poisoning bacteria) produce endospores!!!

FORMATION

- Spore septum begins to isolate newly replicated DNA and a small portion of cytoplasm
- Plasma membrane starts to surround DNA, cytoplasm and membrane isolated in step 1
- Spore septum surrounds isolated portion, forming forespore
- Peptidoglycan layer forms between membranes
- Spore coat forms
- Endospore is freed from cell



Spore = eukaryote, endospore = prokaryote
Ribosomes (all cells contain)

- Information processing, where mRNA is translated to protein.
- Small and large subunits. Show genetic relationships of all cells
- Each has an RNA molecule and r Proteins (also conserved)
- Highly conserved in sequences

Relationships between bacteria

- Similarity of sequences show how closely they are related
- Space species = at least 97% identical in rRNA sequence
- Same genus= at least 93% identical in rRNA sequence
- All bacteria = share at least 70% sequencies identity

Purpose of classification and identification

- To diagnose disease or spoilage organism
- To create a classification system to group like species- unique name – rapid identification
- Makes it easier to recognise new species
- Shows broad relationships between species, explains genetic interaction

Linnaean System (plants and animals) -18th century

- Genus (closely related but non-interbreeding)) + species(similar organisms that can interbreed) = doesn't work in prokaryotes as they are haploid and don't breed

Methods used to classify and identify bacteria

- Morphology – DNA sequence (rRNA gene) (GENOTYPIC)
- Differential staining – mass spectroscopy, PCR, nucleic acid hybridisation. Identifies protein fragments of bacterial cells by their mass
- Biochemical tests – DNA base composition (%G+C), fatty acid profiles (PHENOTYPIC): inoculate chambers with pure culture, detect colour changes from metabolic changes with different substrates, look up a reference key.

- Serology, phage typic, respiration