BIOL1007(MOD 3 - Global Health)

From Molecules to Ecosystems (University of Sydney)

Lecture 18: Microbiology and the 'One Health Concept'

Describe the five major types of microbes	**Viruses**	• Smallest and simplest biological entities
		• **Depends** on the host cell to:
		    ○ Replicate: Needs to be inside the host's cell
		    ○ Metabolism: Steals energy and materials to make more virus particles
		• Have larger effects on other organisms and ecosystems

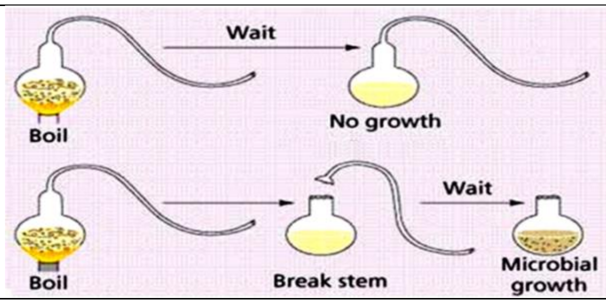
	Bacteria	• Unicellular Structure
		• Smallest **free-living**(live on their own unlike viruses) organism
		• Acquire complex biochemistry and interactions
		    ○ Some can grow on inorganic compounds
		    ○ Organic materials are not necessary for their growth

	Fungi	• Large complex cells → Eukaryotes
		• Can be unicellular
		    ○ Have larger genomes
		    ○ More internal organisation in the cell

	Protists	• Very diverse and difficult to classify ○ In terms of morphology, lifestyles and their evolutionary histories • Large complex cells → Eukaryotes (BUT they are not FUNGI) • E.g. PROTOZOANS: protists that are animal-like → predatory
	Algae	• Unicellular • Large complex cells → Eukaryotes • Complex cell structure → Have chloroplast • They are plant-like protists • Method of metabolism: PHOTOSYNTHESIS
Describe key inventions and ideas in microbiology	**Microscopy**	• 1664 (Robert Hooke): Discovered the structure of blue mould using 30x magnification microscope • 1684(Antonie van Leeuwenhoek): FIRST evidence of bacteria and protists ○ Found out that everything was covered in bacteria

	Agar Plates	• Can be seen down the microscope
		• Used his method to identify:
		  ○ Tuberculosis
		  ○ Cholera
		  ○ Anthrax

	Spontaneous Generation	*"The idea that non-living objects can give rise to living organisms"* **Pasteur disproved this.** Pasteur used 'swan-necked' flasks

		
	Germ Theory	*"Microbes cause diseases"* → Disapproves the idea of 'spontaneous generation
	Koch's postulates	Concluded that an organism that causes a disease must: • Be found in all cases of the disease • Be isolated from the diseased host in pure culture • Produce same disease in experimentally-infected hosy

	Penicillin	• 1928(Alexander Fleming): Found mould growing on a petri dish killed bacteria
		  ○ 'Mould juice' killed many bacteria
		• 1935-1945: Had purified penicillin and developed mass production
		  ○ It was the first effective antib iotic during WWII

Explain the concept of One Health	• A unifying principle for microbiology • Animals, plants and the environment need to be considered as a factor in managing human diseases • Many human disease originate in either the environment or in animals • Use of antimicrobials in agriculture impacts on nhuman pathogens and human health • Disease emergence and spread are influenced by factors such as urbanisation, climate change, pollution etc.	
Describe one major problematic infectious disease	EXAMPLE: **Tuberculosis** ⇒ Caused by a bacterium ⇒ Spreads person to person by airborne droplets → coughing ⇒ Infects lungs → cough, chest pain, weight loss, death ○ Bacteria starts to grow on the lungs ⇒ Can be **latent** AKA have no symptoms ⇒ New strains of tuberculosis are resistant to antibiotic treatment	

Lecture 19: Microbes, Food and Nutrition

Describe the role of microbes in each stage in the food production and consumption chain	**soils, plants, animals**	⇒ **Soil:** Maintain soil health ○ Fixes nitrogen: $N_2 \rightarrow NH^{+}_4$ ▪ Plants use ammonia as a plant source ○ Breaking down organic wastes into inorganic **nutrients** ○ Suppressing animal + plant pathogens ○ Breaking down toxins e.g. pesticides ⇒ **Animals:** Enable to **digest cellulose** (a sugar polymer, abundant in plants, carbon-rich but difficult to digest) ○ RUMEN microbes break down cellulose → sugars → organic acids, CO_2, CH_4 ▪ CH_4 from ruminants → CLIMATE CHANGE: METHANE ○ Organic acids and microbial cells are then digested by animal as nutrients ⇒ **Plants:** Promote plant growth via MUTUALISM ○ Mutualism: ecological interaction where both partners benefit (NOT SYMBIOSIS) ○ Mycorrhizal fungi: Enhances water + inorganic nutrient uptake ▪ In return, receive sugars from plant ○ Rhizobium bacteria: Fixes nitrogen → Gives plants the production of photosynthesis ▪ In return, receive sugars
	Fermentation & Food processing	Two main meanings of 'fermentation': • Microbial transformation of foods by fungi or bacteria → giving value/flavour of • Anaerobic metabolism of sugars → which results in alcohols, acids, CO_2 as end product EXAMPLES of fermentation: • **Barley:** Source of sugars to support fermentation • **Hops:** Flavouring agent → natural preservative → provide bitterness ○ Stops the beer from going bad when other microbes getting into it and start growing • **Yeast:** Ferments sugars to alcohol and CO_2 EXAMPLES of fermented food: beer, wine, bread, kimchi etc.
	Food spoilage	This is due to • GROWTH of **fungi** and **bacteria** • Enzymes that these microbes make/secrete Prevention methods: • Refrigeration • Preservatives • Fermentation
	Food poisoning	Different from FOOD SPOILAGE, the food still looks 'okay' but can be dangerous • **Food-borne infection:** microbes grow in gut • **Food-borne intoxication:** microbes make toxins in food

Provide examples of microbial pathogens in the food supply chain that affect crops, livestock and human health	**Plant Pathogen**
	• Fungi and viruses are the main problems
	• Crop pathogens causes global losses of ~30% of total yield (approx. $1 trillion)
	• E.g. Sigatoka fungi
	    ○ Threatens survival of bananas globally
	        ▪ Modern Cavendish banans are grown from cutting → genetically identical → ALL equally susceptible
	        ▪ Therefore, since they are identical, all of them can get the disease
	Animal Pathogen
	• Inflict suffering, death and massive economic losses (~20% of total production)
	• Viruses, bacteria, fungi or protists
	• E.g. Foot-and-Mouth-Disease (FMD)
	    ○ Infects cows, pigs, sheeps, goats but NOT humans
	    ○ Huge economic losses
	    ○ Cause: Pigs were fed waste products including illegally imported meat from infected animals
	    ○ Poses many ethical and environmental issues in addition to economic problems

Describe the gut microbiome and discuss the role of the gut microbiome in health and disease	• Primarily BACTERIA • We have approx. 40 trillion bacteria in gut microbiome • High fibre diet: Increases Bacteriodetes • High protein and fat diet(more UNHEALTHY): Increases Firmicutes **Healthy Microbiomes are Important For:** • Proper food digestion → breaking down of food • Resistance to pathogens • Immune functioning → distinguish friends from foes • Mental health **Bad microbiome is linked to:** • Allergies • Type 2 diabetes • Cancer • Obesity **EXAMPLE: Gut Microbiome and Obesity** • Obese mice have a distinct gut microbiome • Transplanting ‘obesity-associated’ microbiomes makes germ-free mouse obese

Lecture 20: Planetary Health: Microbes and Ecosystems

<table>
<tr><th colspan="2">Appreciate that the majority of life's diversity is microbes</th></tr>
<tr>
<td>Describe the role of microbes in the carbon cycle</td>
<td>
Most biological processes are done by microbes
<u>Autotroph</u>
<table>
<tr><td>Algae</td><td>

Uses CO_2 as a carbon source
Can use either light or chemical as energy source
Convert inorganic C to organic C, act as "SINKS" for CO_2 → limit climate change

</td></tr>
<tr><td>Methanogen</td><td>

Consume CO_2 and H_2 → produces methane
CO_2 is C source and H_2 is energy source
Impact on climate change: Act as "sinks" for CO_2, but act as <u>sources</u> of CH_4 (BAD)
Breathe CO_2 and exhale CH_4
They are anerobic → killed by oxygen

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<u>Heterotrophs</u>
<table>
<tr><td>Methanotrophs</td><td>
Consume methane, produce CO_2 → It acts as their carbon and energy source

Impact on climate change: act as sinks for CH_4 but act as sources for CO_2 → OVERALL GOOD

</td></tr>
<tr><td>Decomposers</td><td>

Recycle dead cells back to CO_2
SOURCES of CO_2 → bad for climate change

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<tr><td>Predators</td><td>

PROTISTS are PREDATORS but some are 'detrivores'
E.g. amoeba engulfs bacteria

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<tr><td>Pollutant degraders</td><td>
Hydrocarbon-degrading bacteria specialise in eating ancient fossilised organic carbon → contain special enzymes which can attack hydrobcarbons

This is useful for "Bioremediation": Clean up of pollution by microbes

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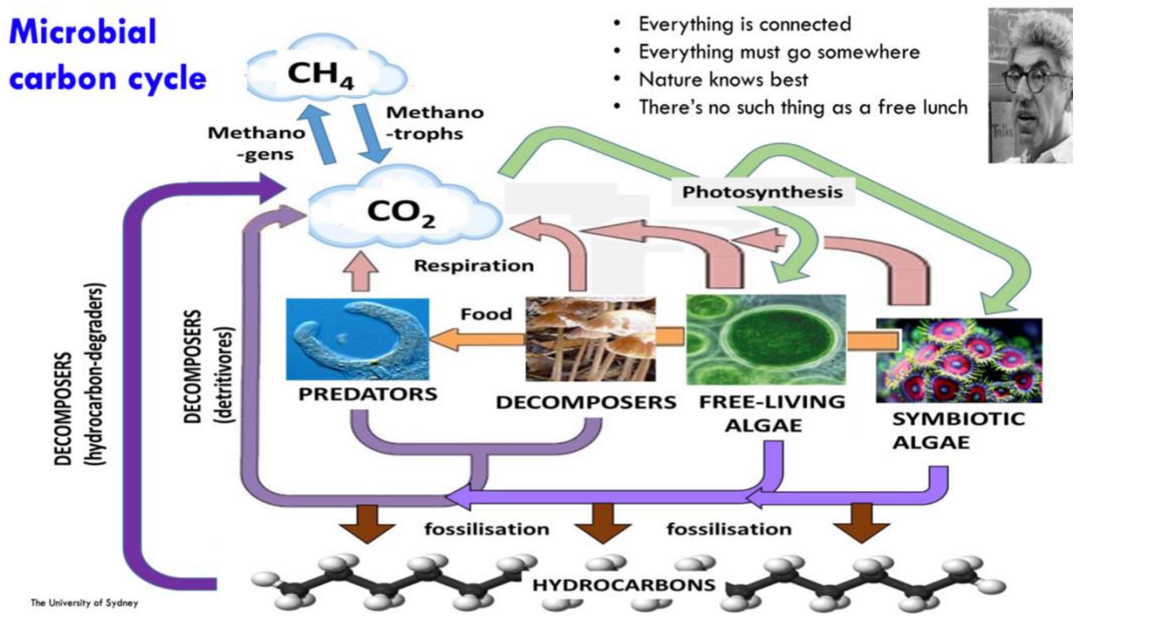

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<tr>
<td rowspan="1">Define an autotroph and a heterotroph, and give microbial examples of each</td>
<td colspan="2">

- **Autotroph:** “self-feeder”
  - E.g. algae and methanogens
- **Heterotrophs:** eating others
  - Eg. Methanotrophs, decomposers, predators, pollutant degraders

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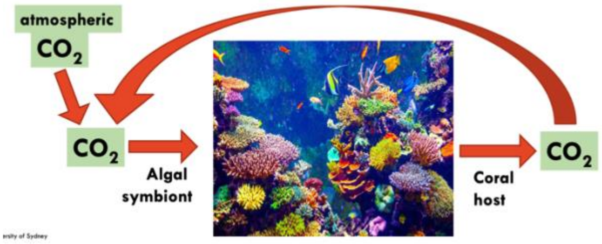
<tr>
<td>Explain the impacts of microbes on global climate as sinks of CO_2 and CH_4</td>
<td colspan="2">Look in the first learning outcome question</td>
</tr>
<tr>
<td rowspan="3">Provide examples of the importance of microbes in:</td>
<td>Marine ecology (coral-algal symbiosis)</td>
<td>

- Corals are primitive animals, which depend on <u>symbiotic</u> microscopic algae to supply them with food
- Algae: Photoautotrophs, convert CO_2 + light → sugars
- Coral: heterotrophs, convert sugars → CO_2



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<td>Terrestrial ecology (lichen symbiosis)</td>
<td>

- **LICHENS** are primary producers in some terrestrial habitats
- Symbiosis between two microbes: A heterotrophic fungus and an autotrophic algae
  - ALGAE: Performs photosynthesis
  - FUNGUS: Provides host for the sytem

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<tr>
<td>Pollution clean-up (bioremediation)</td>
<td>Clean up of pollution by microbes</td>
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Lecture 21: Cell Factories and Biotechnology

<table>
<tr>
<td>Explain why microbes are useful for biotechnology, using examples of specific fungi (S.cerevisiae) and bacteria (E.coli)</td>
<td>
<u>General</u>
<table>
<tr><td>Virus</td><td>Carry genes into new hosts
Source of enzymes (depending on enzyme to manipulate DNA)</td></tr>
<tr><td>Archae</td><td>Source of thermostable polymerase enzymes for copying DNA sequence → VERY STABLE → maintain high enzyme activity at high temperature</td></tr>
<tr><td>Bacteria</td><td>Excellent hosts for cloning DNA and expressing DNA
Use to manufacture things
“FACTORY”
Adopt to new stuff e.g. if they are given new instructions they will do it</td></tr>
<tr><td>Algae</td><td>Conversion of CO_2 + light into biofuels (ethanol, H_2)</td></tr>
<tr><td>Fungi</td><td>Yeasts: Excellent cloning and expression hosts
Moulds: Antibiotic synthesis</td></tr>
</table>
<table>
<tr><th>E.coli</th><th>Saccharomyces</th></tr>
<tr><td>Fastest growth
Very easy to extract or add plasmid DNA</td><td>Better for expressing eukaryote genes
Generally recognized as (GRAS)</td></tr>
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<tr>
<td>Explain what a plasmid is, and define the roles of different kinds of plasmids in nature and in biotechnology</td>
<td>
Plasmid: circular DNA elements found in microbes; replicate independently of the chromosomes

They are the most commonly used vectors for delivery of foreign DNA into a target host cell

‘Wild’ plasmids found in nature allow microbes to swap useful genes → swapping genes without making interactions → HORIZONTAL gene transfer

Key features of plasmids in biotech:

Selectable marker
Enables us to force cells to take up plasmids
Cloning site → A site recognized by the restriction DNA and open it to add a new DNA in
This is where foreign genes can be added
Replication functions → Ensures persistence in host

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<td>Define the terms “DNA cloning”, “recombinant DNA”, “GMO”</td>
<td>

DNA cloning: Making copies of a piece of DNA by adding it into a plasmid, then replicating plasmid

Enzymes included: Thermostable polymerase(copying DNA), restriction enzyme(cutting DNA), T4 ligase(joining DNA)
Steps:

1. Digestion and Ligation
2. Transformation and screening
3. Final Product = GMO
4. Expression of genes(s)

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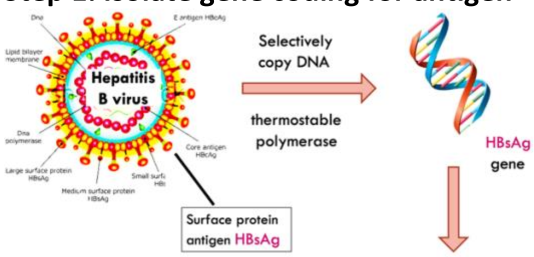
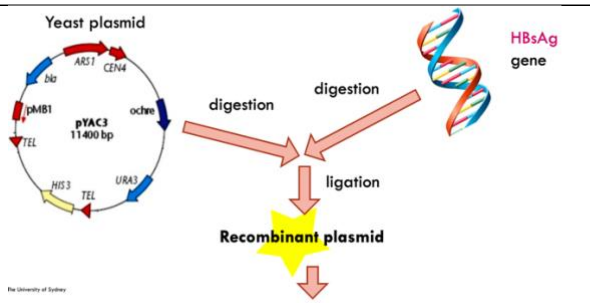
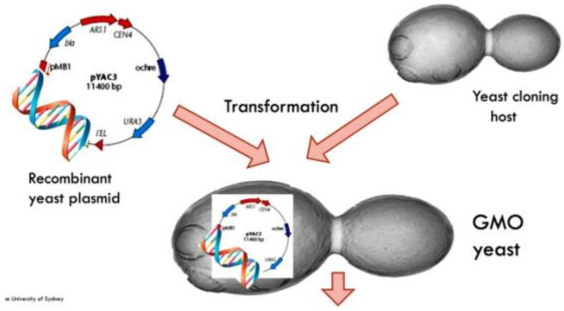
	• **Recombinant DNA:** An artificially made DNA strand that is formed by the combinantion of two or more gene sequences • **GMO(genetically modified organism):** recombinant microbe carrying gene of interest

Explain how recombinant DNA and GMOs are made, and especially which enzymes do which jobs in this process	Made through **DNA CLONING**
	
	Part 1 (Digestion and ligation):
	• *Thermostable polymerase* copies the DNA and the *restriction enzyme* will cut it into pieces → 'DIGESTION'
	• *Restriction enzyme* cuts a section of the plasmid and sticks the pieces of DNA by *T4 ligase* → 'LIGATION'
	• End product: RECOMBINANT (Contain bits of foreign DNA)
	
	Part 2 (Transformation and Screening):
	• The ligation mixture is added into the cloning host → 'Transformation' = uptake of DNA
	• Select plasmid-containing cells e.g. plate on antibiotic agar
	• SCREENING (to find gene of interest):
	    o Looking for DNA directly – Sequence-based screen
	    o Looking fot effect of gene on host – Phenotypic screen
	
	Part 3 (Final Product: GMO)
	
	Part 4 (Expression of genes):
	The promoter goes through the process of transcription and translation to result in a protein

Discuss why vaccines are important, and how recombinant DNA methods can be used to make them	Vaccines:
	• Are a primary defence against infectious disease
	• Lead to 'herd immunity'
	• Work by 'training' the immunse system to recognise antigens, which is associated with an invader
	• Consist of:
	    o Live attenuated microbes
	    o Killed microbes
	    o Antigens(proteins) produced in a GMA host
	    o mRNA coding for antigens
	Example of Hepatitis B vaccine
	Step 1: Isolate gene coding for antigen
	
	Step 2: Cloning Antigen



Step 3: Transformation into Yeast



Step 4: Gene Expression, protein purification