Edexcel (B) Biology A-level

Topic 6: Microbiology and Pathogens

Notes

Microbial Techniques

In a **culture**, microorganisms are provided with the nutrients, level of oxygen, pH and temperature they need to grow in large numbers so they can be observed and measured.

Microorganisms have to be cultured using **aseptic technique**. This involves only introducing the desired bacteria into the medium, under sterile conditions, in order to prevent the growth of unwanted organisms. Even if you think the bacteria are non-pathogenic, there may be a mutant strain present that is pathogenic, or there may have been contamination with a pathogenic microorganism from equipment, air, skin etc.

Aseptic culture technique:

- Decide on the microorganisms you want to culture and obtain the culture.
- Provide microorganisms with **appropriate nutrients** in **sterile nutrient medium**: either broth (liquid) or agar (solid). Most need minerals/nitrogen/carbon. Most also need the medium to be enriched with protein from a blood/yeast/meat extract.
    - Some microorganisms need a very specific combination of nutrients and therefore need to be grown in a **selective medium** (medium containing a very specific balance of nutrients – this means only very specific bacteria will grow in it and mutant strains won't).
- Inoculate the culture.
    - If using broth, either use an inoculating loop and swirl it in the culture or mix inoculating broth with sterile medium.
    - If inoculating agar, either make a **streak plate** (sterilise inoculating loop by flaming, dip in culture, sterile plate, at least three streaks straight or zig-zag, turn, streak which must overlap with first streak, turn, streak to try to obtain single colonies) or a **spread plate** (drop some on and use a sterile spreader to distribute it). If you're looking for single colonies you can then incubate the plate and use an inoculating loop to obtain individual colonies.

Broth can provide anoxic conditions as well as oxygen closer to the surface so can provide information about what kind of oxygen requirements the microbes have. They can also grow a much larger volume of bacteria. However, you can't get a single, discrete, pure colony from a broth to inoculate with/study.

Aseptic technique:

- All equipment, including agar and Petri dishes, should be **sterile**
- Flaming equipment in a **Bunsen flame** ensures sterility
- **Inoculation** should be done with a flamed instrument
- Lids should be replaced as quickly as possible

The growth curve of a microorganism in a closed culture has various distinct features:

1. **Lag phase** - the microorganisms are **adapting to their environment** and reproduction rate increases slowly
2. **Log phase** - microorganisms grow at their **maximum rate**, as long as there are **sufficient nutrients**
3. **Stationary phase** - death rate = reproduction rate (due to build up of waste products and lack of nutrients)
4. **Death phase** - deaths exceed new cell population as conditions continue to deteriorate

Bacterial growth can be measured in a variety of ways:

Cell Count:

- Cells can be counted using a **haemocytometer** (a thick microscope slide engraved with a grid and a rectangular chamber that holds a standard volume of liquid (0.1 mm^3)).
- The sample of broth is diluted 1:1 with **trypan blue**, which stains dead cells blue.
- You count the bacterial cells in each of the four sets of 16 squares and take a mean. The haemocytometer has been pre-calibrated so that the number of bacterial cells = number counted * 10^4 per cm^3. This is repeated at regular intervals throughout growth.
- Cell counting is useful because it counts only viable (living) cells and is accurate. However, it is slow and the equipment involved is expensive.

Turbidimetry:

- Turbid = opaque/cloudy/thick with suspended matter.
- Turbidimetry is a specialised form of **colorimetry**. As turbidity increases, transmission decreases and absorbance (measured in Au, arbitrary absorbance units) increases. This value can be linked to cell count by measuring absorbance of samples with a known cell count (via counting cells with a haemocytometer or using dilution plating), and using a **calibration graph** to obtain the cell count in an unknown sample.
- Turbidimetry is useful because it is quick and can be conducted in the field. However, equipment is expensive; values are affected by other variables, it counts non-viable cells as well as viable, a calibration curve is required to obtain an actual cell count, and it assumes that the agitation and therefore density of cells is equal across the culture (a magnetic stirrer is used to improve agitation).

Dilution Plating:

- Dilution plating works on the principle that every colony is grown from a **single, viable microorganism**. Immediately after culturing, colonies cannot be counted because a single mass is often present. So that single colonies can be seen, the original culture is serially diluted, a lawn plate made and the colonies counted. This is then multiplied by the dilution factor to obtain a cell count.

- Dilution plating is useful because it doesn't require complex or expensive equipment, it only counts viable cells and it obtains a direct cell count. However, it is slow because an **incubation period** is needed and serial dilutions are required.

Bacteria As Pathogens

Bacteria can be agents of infection, via the production of **endotoxins**, **exotoxins** and **host tissue invasion**.

Endotoxins: lipopolysaccharides in the outer lipid membrane of Gram negative bacteria e.g. **Salmonella**. Endotoxins may be released from a bacterium if it breaks down, and have effects local to the site of infection.

Exotoxins: soluble proteins produced and released by bacteria as they metabolise and reproduce e.g. **Staphylococcus**. Exotoxins are spread around the body in blood and body fluids.

An example of a bacterial disease caused by host tissue invasion is **tuberculosis** (TB). TB is caused by a bacteria called ***Mycobacterium tuberculosis*** which infects phagocytes in the lungs:

- First infection is symptomless. Infected phagocytes are sealed in **tubercles** as a result of an **inflammatory response** in the lungs.
- Bacteria lie **dormant** inside the tubercles. They are not destroyed by the immune system as the tubercles are covered with a **thick waxy coat**.
- When the immune system becomes weakened, the bacteria become active again, and slowly destroy the lung tissue, thus leading to breathing problems, coughing, weight loss, and fever.
- TB can be **fatal**.

Action of Antibiotics and Antibiotic Resistance

Antibiotics can also be used to fight infection by killing the bacteria and stopping their growth. There are two types of antibiotics:

- **Bactericidal antibiotics** kill bacteria by destroying their cell wall thus causing them to burst. An example is **Penicillin.**
- **Bacteriostatic antibiotics** inhibit the growth and reproduction of bacteria by stopping protein synthesis and production of nucleic acids so the bacteria can't grow and divide. An example is **Tetracycline,** which interferes with protein synthesis in 70S ribosomes.

However, some bacteria become **resistant** to antibiotics as a result of **natural selection**. The bacteria which are not killed by the antibiotic possess a **selective advantage** – resistance which enables them to survive and reproduce. Therefore, the allele for **antibiotic resistance** is passed onto their offspring, thus creating a **resistant strain.**

Moreover, there is an ongoing **evolutionary race** between organisms and pathogens as **pathogens evolve adaptations** which enable them to survive and reproduce. For instance, the constantly changing protein coat (antigen coat) of HIV means that the virus is not recognised and destroyed by the immune system.

Resistance to antibiotics results in **antibiotic resistant bacterial infections** in hospitals such as **MRSA.** Hospitals have developed various ways of controlling the spread of antibiotic resistant infections, for example:

- New patients are screened at **arrival, isolated and treated** if they are infected to prevent the spread of bacteria between patients.
- **Antibiotics are only used when needed and the entire course of antibiotics is completed** to ensure that all the bacteria are destroyed and to minimise the selection pressure on bacteria to prevent resistant strains from forming.
- All staff must follow the code of practice which includes **strict hygiene regimes** such as **washing hands with alcohol-based antibacterial gels** and wearing suitable clothing which **minimises the transmission of resistant bacteria.**

Other Pathogenic Agents and Problems of Controlling Endemic Diseases

Virus: *Influenza*

Transmission: **droplet infection**, direct contact with **virus-filled mucus**, direct contact with animal waste (zoonotic infection), direct contact with infected surfaces.

Mode of infection: infects **ciliated epithelial cells** of the lungs (antigen fits into receptor on host cell, injects viral RNA), viral RNA takes over cell biochemistry, cell produces new virus particles, cell lyses, many virus particles released.

Pathogenic Effects: headache, sore throat, coughing, sneezing, muscular/joint pain, vomiting, fever etc. lasting about 5-7 days

Treatment/Control: antiviral medication, antibiotics for secondary bacterial infections, treatment of symptoms e.g. painkillers

Fungus: *Puccinia Graminis* (Stem Rust Fungus)

Transmission: **wind carries spores** from infected plants, infected fragments left in soil from the two hosts – cereal crops and *Berberis.*

Mode of Infection: spore germinates in water on plant, produces **hyphae** which enter the plant through the stomata, hyphae grow into **myecelium**, surround all tissues in the plant, produce enzymes e.g. cellulase to digest the plant and nutrients are absorbed into the fungus.

Pathogenic Effects: nutrients lost to the fungus, weakened stem, water loss as the plant can't control transpiration (reduced photosynthesis), pustules on epidermis which eventually burst to release more spores.

Protozoan: *Plasmodium spp.* Malaria

Transmission: transmitted through the vector of the **female *Anopheles* mosquito** when she feeds to get protein to lay eggs.

Mode of Infection: parasite transmitted via mosquito, travels to liver, infects red blood cells, reproduces asexually inside erythrocytes and causes lysis.

Pathogenic Effects: Malaria bursts out of red blood cells every 2-3 days, causing paroxysm, sweating, shaking, muscle pains, headaches, liver damage, anaemia.

Plasmodium spp reproduce **sexually** in mosquitoes and **asexually** in red blood cells in humans.

Treatment/Control: mosquito nets (especially LLINS –50% more effective), insect repellent, pesticides, mosquito screens, more clothing, avoiding standing water, treating standing water with pesticides to remove mosquito larvae, proper disposal of sewage, introducing predators for mosquitoes. Quinine, chloroquinine and artemisinin are antimalarial drugs that work best in combination. Accurate diagnosis via observation with a microscope.

Treatment/Control:
Controlling **endemic diseases** is very difficult because:

- The disease is often widespread
- Difficult to remove all sources of infection
- Expensive to provide treatment

Preventing mosquito bites:

- Insect repellents, mosquito nets with insecticides, screens on doors and windows, clothing to cover skin

Controlling mosquito numbers:

- Avoid standing water and sewage, water treatment to kill larvae, biological control by introducing predators

There are many **ethical, social and economic** implications of control methods.

Ethical implications:

- Informed consent may be difficult
- Spraying mosquitoes with insecticides will affect other organisms

- Money spent on vaccines could instead be spent on education/preventing famine

Social implications:

- Vaccines need to become accepted
- Social changes to reduce infection are difficult to bring about

Economic implications:

- Treatment, control and prevention of endemic diseases is very expensive
- Malnutrition may be more of a threat to human life than malaria

Response to Infection

Physical barriers to infection include:

- **Skin** is a **tough physical barrier** consisting of **keratin**
- **Stomach Acid** (hydrochloric acid) which **kills bacteria**
- **Gut and skin flora –** natural bacterial flora **competes with pathogens** for food and space

Non-specific responses of the body to infection include:

- **Inflammation –** histamines released by white blood cells cause vasodilation, which increases the flow of blood to the infected area and increases permeability of blood vessels. As a result, antibodies, white blood cells and plasma leak out into the infected tissue and destroy the pathogen.
- **Fever –** the hypothalamus sets the body temperature higher. This decreases speed of pathogen reproduction and increases rate of specific immune response.
- **Lysozyme action –** lysozyme is an enzyme found in secretions such as tears and mucus which kills bacterial cells by damaging their cell wall.
- **Phagocytosis** is a process in which white blood cells engulf pathogens and destroy them by fusing a pathogen such as bacteria enclosed in a phagocytic vesicle (phagosome) with a lysosome.

The specific immune response is antigen specific and produces responses specific to one type of pathogen only. This type of immune response relies on **lymphocytes produced in the bone marrow**:

- **B cells** mature in the bone marrow and are involved in the **humoral immune response.**
- **T cells** move from the bone marrow to the thymus gland where they mature, and are involved in the **cell mediated immune response.**

Cell-Mediated Response

1. Pathogen invades a host cell.
2. The host cell displays the antigens on its **Major Histocompatibility Complexes** and becomes an **Antigen-Presenting Cell**.
3. **T Killer cell** with complementary receptor proteins binds to the APC.
4. **Cytokines** secreted by active T Helper cells stimulate the T Killer cell to divide by mitosis.
5. T Killer cell divides to form **active T Killer cells** and **T Killer Memory cells.**
6. Active T Killer cells bind to APCs and secrete chemicals which cause pores to form in the cell membrane.
7. The infected cell dies.

Humoral Response

T Helper Activation:

1. Bacterium is engulfed by a **macrophage**. Surface antigens are passed along the endoplasmic reticulum into a vesicle which are transported to the cell surface membrane.
2. Macrophage acts as an APC and presents antigens on MHCs.
3. Macrophage APC binds to T Helper cell with **complementary receptor proteins**.
4. The T Helper cell is 'activated' and divides by mitosis to form T memory cells and active T helper cells.

Effector Stage:

1. Antigens from APCs that are **complementary to the antibodies on B cells** bind and are taken in by endocytosis.
2. The B cell acts as an APC and presents antigens on MHCs.
3. An activated T helper cell (from the previous stage) with a complementary receptor protein to the antigens binds to the APC. It produces **cytokines**.
4. Cytokines stimulate the B cell to divide by mitosis and form **B memory cells** and **B effector cells**.
5. B effector cells differentiate into **plasma cells**.
6. Plasma cells synthesise antibodies.
7. Effects of antibodies:
    a. Agglutination (microbes clump together – makes phagocytosis easier)
    b. Lysis (bursting of bacterial cells)
    c. Opsonisation (antibodies coat microbes and mark them for phagocytes)
    d. Precipitation/Neutralisation (soluble toxins are made insoluble)
8. T Suppressor cells stop the immune response.

Immunity can either be **active or passive**; active **immunity results from the production of antibodies by the immune system** in response to the presence of an antigen whereas

passive immunity results from the **introduction of antibodies from another person or animal.**

There are also two subtypes of immunity; natural or artificial:

- **Natural active immunity** arises from being exposed to an antigen/getting the disease whereas **natural passive immunity** is the result of crossing of mother's antibodies through the placenta and their presence in breast milk.
- **Active artificial immunity** is acquired through vaccinations which stimulate the immune system and lead to production of antibodies whereas **passive artificial immunity** is where antibodies are injected into the body.

Herd Immunity = enough people have been vaccinated to make transmission of a disease very unlikely. Requires 80-90% vaccination. Immunisation is the process of protecting people from infection with passive/active artificial immunity – vaccination is the process by which this is achieved through the use of **attenuated antigens.**

- The secondary infection has **less lag** (so less time for symptoms), is more rapid and produces more antibodies and T Killer cells than the primary response because there are Memory T and B lymphocytes in circulation from the primary infection.