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 to the medium. 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 microorganisms you want to culture, obtain 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 e.g. 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 take off 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.

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

- The first phase of microorganism growth is the **lag phase,** where microorganisms are **adjusting to the environment** before starting to reproduce. This means that the population doesn't increase at maximum rate during the lag phase.
- The next part of the growth curve is the **log phase,** where the population size grows **exponentially** meaning that **every round of division doubles the population size,** so long as the dividing organism has a **sufficient amount of nutrients**.

- The stationary phase is where the population size reaches its **maximum, due to decreasing nutrient levels** and build of up **toxic substances**. The population doesn't change as reproduction is equal to bacterial death.

- The stationary phase if followed by a **death phase,** where lack of nutrients and increase in toxic products causes **death of organisms**.

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 having 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 so number can be calculated. 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 cell count that is known (via counting cells with a haemocytometer or using dilution plating).
- 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 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 membrane, which forms part of the cell wall of gram negative bacteria e.g. **Salmonella**.

Exotoxins: soluble proteins produced and released by bacteria as they metabolise and reproduce e.g. **Staphylococcus**.

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 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, and weight loss, as well as 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.
- **Bacteriostatic antibiotics** which inhibit the growth of bacteria by stopping protein synthesis and production of nucleic acids so the bacteria can't grow and divide.

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 their course 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), contact with infected surfaces (formites)

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

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

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

Fungus: *Puccinia Graminis* Stem Rust Fungus

Transmission: **spores** via wind/infected fragments left in soil from the two hosts – cereal crops and *Berberis*

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

Pathogenic Effects: absorbed nutrients, 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, infect red blood cells, reproduce asexually inside erythrocytes and cause lysis.

Pathogenic Effects: paroxysm, sweating, shaking, muscle pains, headaches, liver damage, anaemia.

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 mosquitos. Quinine, chloroquinine and artemisinin are antimalarial drugs that work best in combination. Accurate diagnosis via observation with a microscope.

Issues with treatment/control: ethical (difficulty obtaining informed consent, money could be more effectively used, insecticides effect other organisms), social (changing customs), economic (very expensive, countries are very poor, bigger threats), practical (endemic to many countries – widespread, two hosts and complex life cycle, expensive, high antigenic variability, shielded from immune response inside cells)

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 damaged white vessels cause vasodilation, which increases the flow of blood to the infected area and increases permeability of blood vessels. As a result of that antibodies, white blood cells and plasma leak out into the infected tissue and destroy the pathogen.
- **Fever –** the hypothalamus sets 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 thus destroying them by fusing a pathogen such as bacteria enclosed in a phagocytic vacuole 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 response.**
- **T cells** move from the bone marrow to the thymus gland where they mature, they are involved in **cell mediated response.**

Cell Mediated Response

Pathogen invades a host cell.

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