2017 Bacteria and Viruses STQ MS

2017 / H2 / ACJC PRELIM / P2 Q8 (BE)

1 Speciation events have been observed to occur very frequently in bacteria. It was suggested that the high rate of speciation is due to the high level of variation in bacteria.

(a) Transformation and conjugation are two processes which increase the level of variation in bacteria.

Distinguish these two processes.

	Conjugation	Transformation
Genetic material being transferred	**1. Transfers the F plasmid or R plasmid**	**1. Transfers fragments of the bacterial chromosome;**
Donor cell	**2. Donor cells are F^+ cells / carries the F plasmid or R plasmid;**	**2. Donor cells may be lysed cells that releases its DNA;**
Recipient cell	**3. Recipient cells are F^- cells / do not carry the F plasmid or R plasmid;**	**3. Recipient cells must be competent / secrete the competence factor;**
Physical contact	**4. Direct physical contact required between two cells through sex pilus;**	**4. No physical contact required between cells / exogenous DNA taken up directly by recipient cell;**

[3]

Bacterial evolution is one of the most dynamic and exciting areas in current biological research.

Over the years, a barrier in this field of research is the difficulty in classifying bacterial species. However, in recent times, new analytical tools in molecular biology have offered new insights into the classification of bacterial species.

(b) (i) Suggest why scientists had difficulties in the classification of bacterial species.

1. Bacteria reproduce asexually / by binary fission;
2. Unable to determine between species according to biological species concept / bacteria are unable to interbreed to produce fertile viable offspring;
3. Horizontal gene transfer/transformation/conjugation between relatively distantly related bacteria / different bacterial species (OWTTE);
4. Results in high rates of recombination, making it difficult to determine a single species;
5. Different species may be morphologically similar;

[2]

(ii) Explain how analytical molecular tools have helped overcome this barrier in research.

1. Molecular tools have helped determine genetic sequences of bacteria / compare genetic sequences of bacteria;
2. Allowing scientists to classify bacteria according to genetic distance / phylogenetic distance;
3. Provides an objective method (to determine genetic distance);
4. Data obtained is quantitative;
5. Hence more sensitive to differences between species / data could be used for statistical analyses;

[2]

(c) The outer layers of the two types of bacteria with peptidoglycan cell walls known as Gram-positive and Gram-negative bacteria are shown in Fig. 8.1 below.

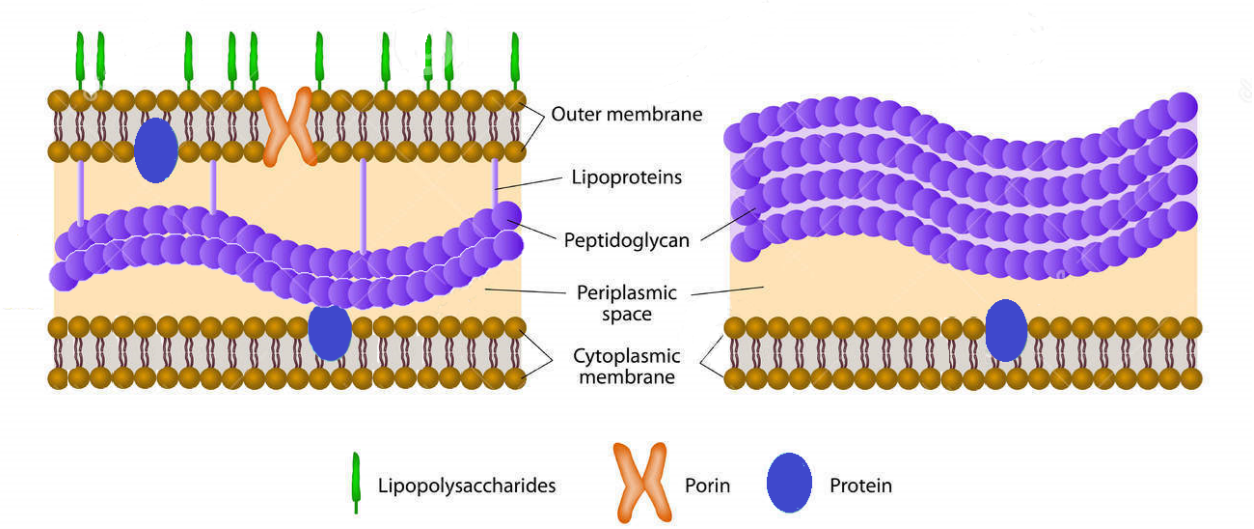


Fig. 8.1

Penicillin is an antibiotic that is known to be effective against only one of the two types of bacteria above.

With reference to the information given and your own knowledge, deduce which type of bacteria is susceptible to the action of penicillin and explain why.

1. Penicillin is effective only against gram-positive bacteria;
2. Gram-negative bacteria have an outer membrane (and lipoproteins) (that surrounds the peptidoglygan layer of the cell wall) / penicillin can directly access the peptidoglycan cell wall in gram-positive bacteria;
3. Preventing the action of penicillin as penicillin inhibits the crosslinks in peptidoglycan cell wall; [3]

(d) Explain how antibiotic-resistant bacteria can become increasingly common in a population of bacteria.

1. **Horizontal gene transfer (transformation, transduction, conjugation) occurs which increases genetic variation in the bacteria;**
2. **Genetic variation exists in the form of antibiotic sensitivity and antibiotic resistance;**
3. **Antibiotics act as selection pressure;**
4. **Non-resistant bacteria are selected against / bacteria which are resistant to antibiotics are selected for / they have a selective advantage;**
5. **Allele coding for antibiotic resistance passed down to subsequent generations of bacterial cells (during binary fission);**
6. **Over many generations, frequency of allele coding for antibiotic resistance increases in the gene pool;**

[4]

[Total: 14 m]

2017 / H2 / AJC PRELIM / P2 Q5

5 Figure 5.1 represents a bacteria DNA and a eukaryotic chromosome in metaphase of mitosis, not drawn to scale.

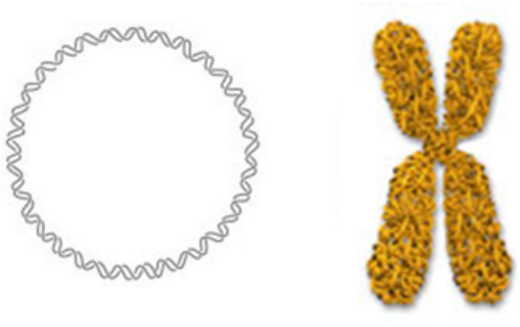


Fig. 5.1

(a) State **two** ways in which the organization of genes found in these two structures differ and suggest **one** advantage of this to the bacterium.

Feature	Eukaryotic	Prokaryotic
Gene organization	Monocistronic genes	Polycistronic genes / **operons**
Advantage to bacteria	Simultaneous expression of closely-related genes organised in an operon	
Association between DNA and histones	Association with **histones** / scaffolding Proteins - allows for increased structural complexity/folding to higher degree of condensation e.g. between euchromatin and heterochromatin states	No association with histones - does not achieve same level of condensation complexity as eukaryote

[3]

(b) In 1946, Joshua Lederberg and Edward Tatum proposed that bacterial cells undergo genetic recombination. To test their hypothesis, they experiments using two bacteria strains of *Escherichia coli (E.Coli*) with different nutritional requirements.

Strain A, B and a mixture of both strains were grown on culture plates containing minimal medium that does not contain essential amino acids. The results are shown in Fig. 5.2.

Mutant genes (–) do not code for enzymes that synthesize amino acids. Note that all five amino acids are required for bacterial growth.

Bacterial strains	Genes for biosynthesis of amino acids	Mutant genes for biosynthesis of amino acids
A	thr^+ leu^+ thi^+	met^- bio^-
B	met^+ bio^+	thr^- leu^- thi^-

Fig. 5.2

Another researcher, Bernard Davis also worked with the same hypothesis. In his experiment he constructed a U-tube in which the two arms were separated by a fine filter. The pores of the filter were too small to allow bacteria to pass through but large enough to allow easy passage of the fluid medium, any dissolved substances and free DNA. The results are shown in Fig. 5.3.

Fig. 5.3

(i) Using the results of the two experiments and your understanding of genetic recombination in bacteria, state the genetic recombination that has taken place between Strain A and B. Explain your answer.

- Conjugation;
- Second experiment shows transduction and transformation did not take place;
- Because no colonies grew on minimal medium agar;
- genetic recombination occurs only when physical contact is possible between 2 strains;
- In first experiment, bacteria that grew on minimal medium has DNA that encodes for all essential amino acids/ ref. recombinant bacteria has grown on minimal medium
- Showing the genes from Strain A have transferred to Strain B / converse through formation of conjugation tube/ ref. to conjugation tube [6]

(c) In 2016, a pathogenic strain of *E.Coli* found on unwashed salad caused food poisoning in 151 people in Britain, leaving two of them dead.

Using a named example, describe how such pathogens are usually treated. [3]

- Antibiotics
- Penicillin
- Competitive inhibitor to transpeptidases, prevent cell wall synthesis;
- Resulting in bacterial cell lysis;

[Total: 12 m]

2017 / H2 / CJC PRELIM / P2 Q8 (ETA)

3 Transpeptidase is a bacterial enzyme that cross-links cell wall peptides during the formation of bacterial cell walls. The antibiotic penicillin inhibits the activity of transpeptidase. Fig. 8.1 shows part of each of the molecular structures of a cell wall peptide and penicillin.

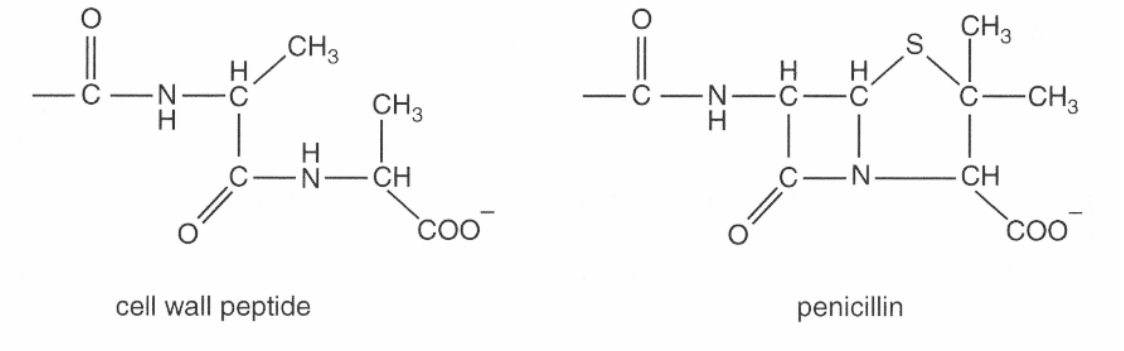


Fig. 8.1

(a) Comment on the structure of cell wall peptides and penicillin.

ANS [L1] (Novel)
[1]

2] Shape **similar configuration** **ring structure**

1. Both have **similar functional groups** C=O [Carbonyl] and COOH [carboxylic groups]
2. Both have **similar shape / configuration**

(b) Suggest why the penicillin molecule is an effective inhibitor of transpeptidase.

ANS [L2] (Adapted from H2 JJC/ 2017 MYE/ Q4bii)
[2]

1. As the penicillin molecule/competitive inhibitor is **structurally similar** to the **cell wall peptide/actual substrate**, the penicillin molecule can **enter and bind/competes** with the cell wall peptide for binding at the **active site** of the transpeptidase;;
2. When the penicillin molecule is bound at the transpeptidase's active site, it **prevents the cell wall peptide** from **entering the site**, preventing the **formation of E-S complexes** and **formation of products**, hence **decreasing the rate of reaction**.

(c) Fig.8.2 shows an electron micrograph of an alveolar macrophage isolated from a tuberculosis patient.

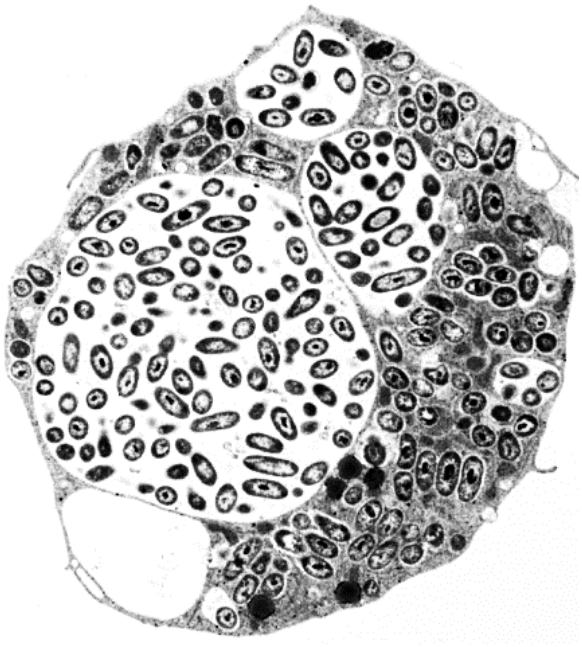


Fig. 8.2

(i) Describe the mode of transmission of Mycobacterium tuberculosis.

ANS [L1] (Novel)
[1]

1. Mycobacterium tuberculosis is carried through **air** in the **infectious droplets** produced when **individual with active TB** cough/speak/sneeze/spit.

(ii) Explain the appearance of the alveolar macrophage in Fig. 8.2.

ANS [L2] (Novel)
[2]

1. **Many M. tuberculosis in the macrophage**. These bacteria were taken into the macrophage via **phagocytosis**.
2. In the macrophage, M. tuberculosis **prevents fusion** of the **phagosome with lysosome**. The bacteria are able to **survive and divide** within the macrophage.

(d) Tuberculosis patients are commonly treated with antibiotics, isoniazid and rifampicin. Recently, there is an increase in number of multi-drug resistant tuberculosis cases.

State **one** reason why multi-drug resistant tuberculosis continues to emerge.

ANS [L1] (Novel)
[1]

1. Inappropriate or incorrect use of antimicrobial drugs.
2. Use of ineffective formulations of drugs.
3. Premature treatment interruption.

[Total 7]

2017 / H2 / DHS PRELIM / P2 Q4

Question 4

(a) (i) Transformation

(a) (ii)
- It is a negative control
- to show that bacteria with no ampicillin resistance gene will not grow / multiply

(a) (iii)
- bacteria strain is not competent

(b) (i)
- The concentration of tryptophan synthesising enzymes deceases while that of lactose utilisation enzymes increases
- The concentration of lactose utilisation enzymes plateau at a higher level than tryptophan synthesizing enzymes

(b) (ii)
- Tryptophan binds to trp repressor proteins and activates it
- Trp repressor protein binds to the operator region on the trp operon and turns off the operon.
- Existing tryptophan enzymes are degraded by enzymes

(c)
- Permease. ;
- So that the lactose, when present, can be transported into the cells to induce the lac operon and turn on the transcription of more lactose utilisation enzymes. ;

2017 / H2 / JJC PRELIM / P2 Q3

1 Antibiotic resistance is rising to dangerously high levels in all parts of the world. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning and gonorrhoea – are becoming harder, and sometimes impossible, to treat as antibiotics become less effective.

Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant either by a genetic mutation or by acquiring resistance from another bacterium.

(a) Outline the process of how a bacterium is able to acquire resistance from another bacterium. [3]

1. **bacterial conjugation;;**
2. **F^+ cell/donor bacterial cell with F factor produces <u>sex pilus</u> to <u>attach</u> itself to F^- cell/recipient cell;;**
3. **A temporary cytoplasmic mating bridge is formed <u>between</u> the two bacterial cells which allows F^+ cell to <u>transfer its F plasmid containing the antibiotic resistance gene</u> to the F^- cell (by rolling circle mechanism);;**

Or

4. **bacterial transformation;;**
5. **A bacterium <u>takes up foreign DNA containing antibiotic resistance gene</u>;;**
6. **The foreign DNA is <u>incorporated into bacterium's own DNA via homologous recombination</u>/<u>through crossing over with a homologous region</u> found on the bacterial chromosome;;**

More than 2 million Americans each year are infected by antibiotic-resistant bacteria, and at least 23,000 die annually from those infections. Antibiotic-resistant bacteria have become a global health crisis and alternative treatments such as Phage Therapy are being considered for combating bacterial infections.

Phage Therapy involves the targeted application of bacteriophages that, upon encounter with specific pathogenic bacteria, can infect and kill them. Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics.

Fig. 3.1 is an electron micrograph showing a phage infecting a bacterium during Phage Therapy.

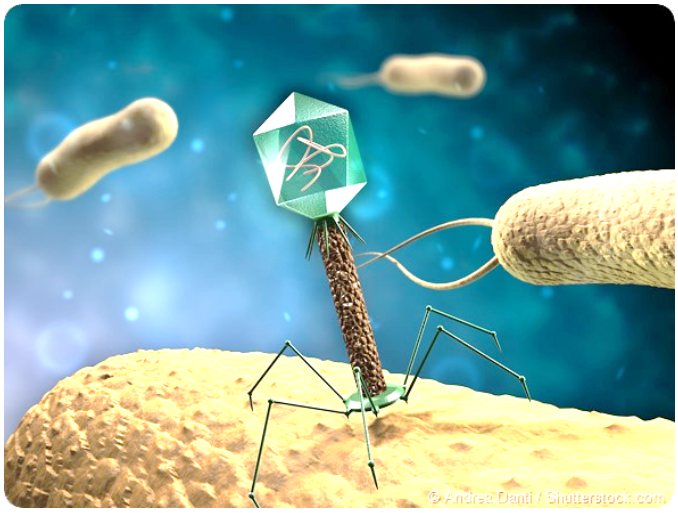


Fig. 3.1

(b) Suggest the reproductive cycle of a phage used for Phage Therapy. [1]

- **lytic cycle**

(c) Describe how a structural feature of the phage allows for targeted application to specific pathogenic bacteria. [2]

1. **attachment sites on its tail fibres;;**
2. **<u>complementary in shape to specific receptor sites</u> on the specific host bacterial cell wall, <u>recognise and adsorb</u> to specific receptor sites on the specific host bacterial cell wall;;**
   (mark for 'specific receptor sites on the specific host bacterial cell wall' once)

(d) Explain how the use of phages can prevent the spread of bacterial infection. [2]

1. **enzymes coded by the genome of the phage shuts down the bacterium's macromolecular (i.e. DNA, RNA and protein) synthesis;;**
   OR
2. **phage nucleases hydrolyse the bacterial chromosome;;**
   OR
3. **lysozyme breaks down peptidoglycan cell wall;;**
4. **new bacteria cells cannot be synthesized;;**
5. **lysis (death) of host cell occurs upon the release of new phage particles;;**

(e) Suggest characteristics of phages that make them attractive therapeutic agents. [2]

1. **highly specific / more specific than antibiotic;;**
2. **very effective in lysing targeted pathogenic bacteria;;**
3. **typically harmless;;**
4. **will not develop resistance;;**
5. **rapidly modifiable to combat the emergence of newly arising bacterial threats;;**

[Total: 10]

2017 / H2 / MJC PRELIM / P2 Q5

QUESTION 6 In May 2014, the Middle East respiratory syndrome coronavirus (MERS-CoV), which was first reported in Saudi Arabia in 2012, infected two Americans who travelled to Saudi Arabia.

Coronaviruses are enveloped RNA viruses that infect and cause lower respiratory tract disease in a broad array of animals and humans. Virus particles range from 70 to 120 nm in diameter and are surrounded by characteristic spike-shaped glycoproteins, as shown in Fig. 5.1. Coronaviruses contain the largest single-stranded, positive-strand RNA genomes currently known, which range from 25.5 to nearly 32 kb in length.

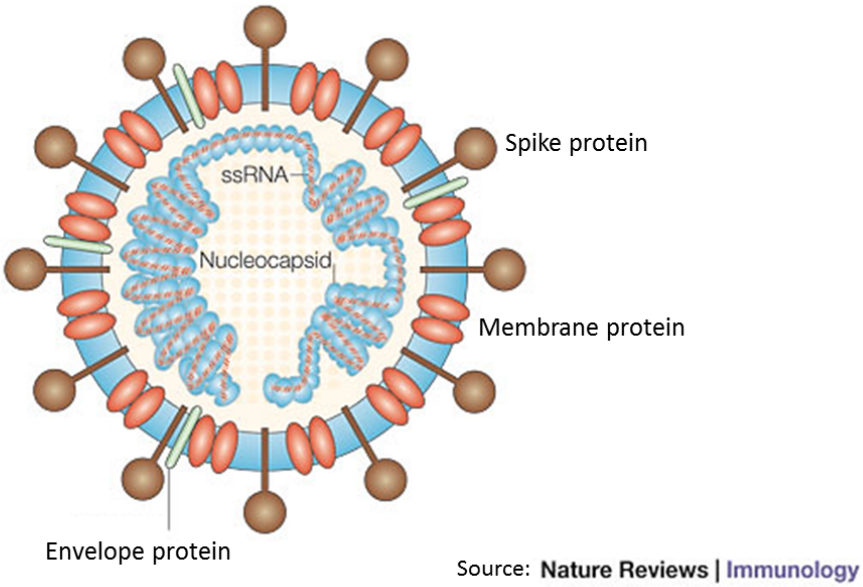


Fig. 5.1

(a) Describe **two** structural differences between the genome of the coronavirus and the influenza virus. [2]

- Genome in influenza virus is **negative-strand RNA**, while that in coronavirus is **positive-strand RNA**.
- **Eight**, **separate** single-stranded RNA in influenza virus, while there is only **one** continuous long RNA strand in coronavirus

(b) Describe how the coronavirus enters its host cell. [3]

[Entry by fusion]

- Spike protein / Glycoprotein is **complementary** in shape to certain cell surface **receptor** on the host cell.
  [Reject if the idea that the receptor is on the host cell is unclear/not present]

- Binding triggers conformational change to the viral envelope protein which in turn result in the **fusion of the viral envelope** with the **host cell surface membrane** is triggered.

- The **nucleocapsid**/**RNA genome released** into the cytosol.

OR

[Entry by RME]

- Spike protein / Glycoprotein is **complementary** in shape to certain cell surface **receptor** on the host cell.
  [Reject if the idea that the receptor is on the host cell is unclear/not present]

- Virus then enters the host cell via **receptor-mediated endocytosis,** where the host cell membrane forms an **endosome/endocytotic vesicle** around the virus.

- **Fusion** of the **viral envelope** with the **endosomal membrane** releases the nucleocapsid/RNA genome into the cytosol.

(c) Describe the process which allows the coronavirus to infect a *broad array of animals and humans* overtime. [2]

- Antigenic drift occurs: **Gene** coding for **spike protein/glycoprotein** undergoes **mutation**.

- **Changes in conformation** to the spike protein which can **bind to various receptors** of different cell types / species.

(d) Unlike the human immunodeficiency virus, the coronavirus genome is not integrated into its host DNA.

Suggest how the coronavirus produces more copies of its genome. [2]

- **(+)RNA** acts as a **template** to produce **(-)RNA**, which in turn acts as a **template** to produce many copies of the (+)RNA genome…

- …by viral **RNA-dependent RNA polymerase**.

[catalysed by replicase, accept if student mention viral RNA-dependent RNA polymerase]

(e) The fatality rate of coronavirus infections is approximately 60%.

Briefly explain how the coronavirus can cause death in humans. [1]

- Since CoV infects and **damages epithelial cells** of the lower **respiratory tract** → **Suffocation** / **respiratory failure leading to death**.

[Total: 10]

2017 / H2 / MJC PRELIM / P2 Q6

QUESTION 7

(a) Fig. 6.1 is an electron micrograph of a process that bacterial cells undergo which results in the formation of two daughter cells.

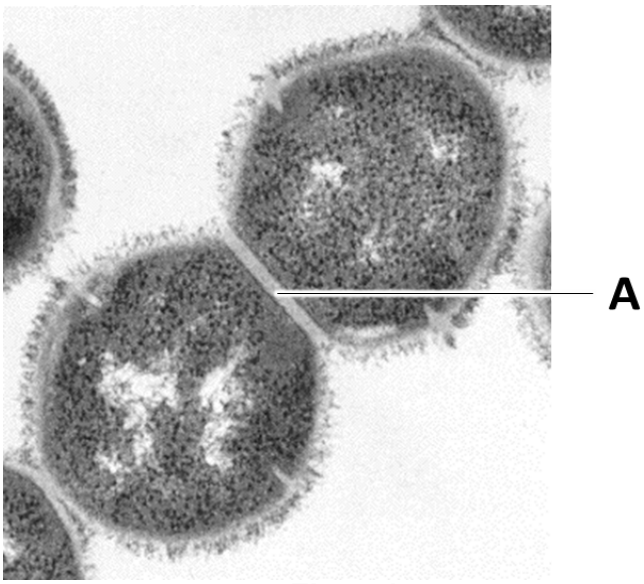


Fig. 6.1

(i) Name the process above and state the main component making up structure **A**. [1]

Process: **Binary fission**

Component making up Structure **A**: **Peptidoglycan** cell wall

[Both correct – 1m]

(ii) "*The process above will always produce two genetically identical daughter cells*".

Comment on the validity of this statement. [1]

[Not always true]

- **No mechanism for equal division** of **plasmids** during binary fission, hence plasmids may not be equally divided between daughter cells.
- **No formation of spindle fibers** in prokaryotes to **pull chromosomes to opposite poles** of the cell, hence may result in unequal division of chromosome.

[True]

- ***Idea that*** Bacterial chromosome has a point of **attachment to the bacterial membrane** which ensures equal separation of bacterial chromosomes during binary fission.

(b) The *xyl* operon is a catabolic operon involved in the breakdown of the sugar xylose. Fig. 6.2 shows how a *xyl-lac* fusion operon is constructed, which consist of 2 structural genes from *lac* operon, regulatory sequences and the regulatory gene of the xylose operon. The arrows indicate the direction of transcription.

To test its effects, the fusion operon was constructed and packaged into bacteriophages. The fusion operon was then inserted into the chromosomes of these bacterial cells upon infection.

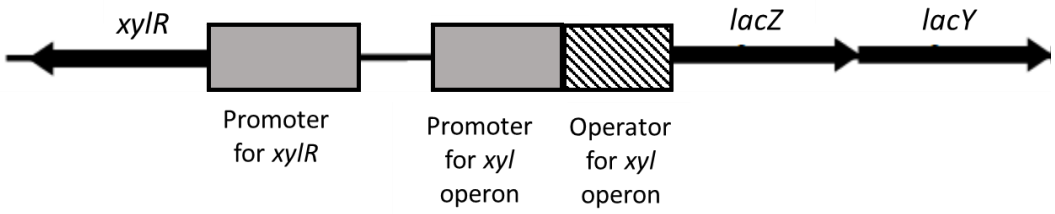


Fig. 6.2

(i) State the process of this gene transfer. [1]

- **Specialised Transduction**

(ii) Suggest and explain one advantage of the process stated in **(i)** over transformation in bacteria. [2]

- In transduction, the operon can be **integrated into the chromosome** of bacteria but it may not be integrated in transformation.
- ***Idea that*** Hence operon replicate when bacteria chromosomes replicate, hence all daughter bacterial cells possess the operon.
  OR
- ***Idea that*** Hence not easily degraded, ensuring more stable expression.

- In transduction, **operon is packaged into phage** capsid but in transformation, operon remains as naked DNA.
- ***Idea that*** Hence phage capsid protects the operon from degradation (by nucleases outside the bacteria cells)

- The **rate of successful gene transfer is higher** in transduction than in transformation.
- ***Idea that*** Phages target bacterial cells specifically / by nature.

(iii) Explain the condition required for *lacZ* gene to be expressed in bacteria cells in which the *xyl-lac* fusion operon has been introduced. [3]

- **Presence of xylose**

- ****Xylose** act as **inducer**, which bind to and inducing a **conformation change** to **inactivate** xyl **repressor**.

- Inactive repressor **cannot bind to operator**, allowing **RNA polymerase** to **bind to promoter** to transcribe the *lacZ* gene.

*Award max 1m (3rd point only) if student identified presence of lactose.

(iv) Suggest why the direction of transcription of the regulatory and structural genes may differ. **KU-3**
[2]

- ***Idea that*** Different strands was used as **template** for transcription.
- Since the strands (used) are **antiparallel**, the two strands are **read in 3'→5' direction in opposite orientations**.

(c) Colibacillosis is a fatal condition caused by *E. coli* in poultry. In a study to examine the effectiveness of bacteriophages in treating colibacillosis, broiler chickens were first subjected to an aerosol spray containing bacteriophages *on day 0*. They were then separated into five treatment groups. Each treatment group was subsequently injected with *E.coli* on days 0, 1, 2, 3 and 4 respectively. The mortality rate for each treatment group was determined after 21 days. The result of the study is represented by Fig. 6.3 below.

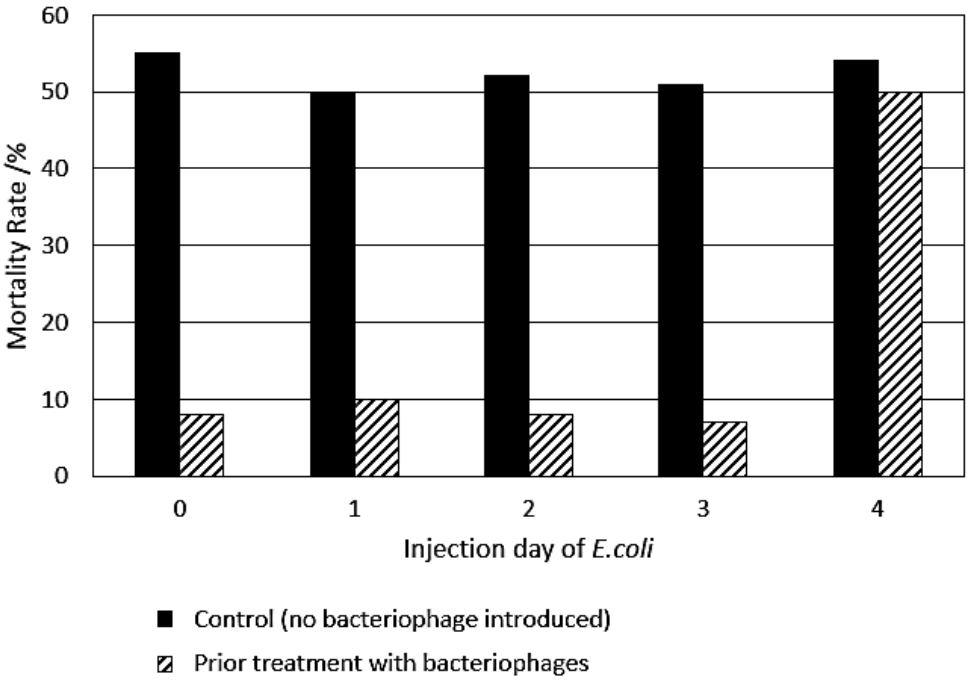


Fig. 6.3

With reference to Fig. 6.3 above,

(i) Compare the trends observed in the control group and the groups that have been treated with bacteriophages, and comment on the effectiveness of such treatment. [3]

[max 2]

- For injection days 0 – 3, the mortality rate for control group is **consistently high**, but **low** for treatment group.
- Mortality rate for control group ranges from 50-55% / averages 53 % (accept 52%) while mortality rate for treatment group ranges from 7(8)–10% / averages 8% (accept 9%).
- On Day 4, there is a spike in mortality rate for the treated chicken to 50% which is **comparable** to that of control which is 53%

(Accept and award pt 1 &/or 2 if students compared day 0-3 individually)

- **[compulsory]** Bacteriophages is effective in reducing mortality rate caused by colibacillosis for **only 3 days** before another dose is needed. **[1]**

(ii) Suggest why the use of bacteriophages is a better alternative to antibiotic therapy for the chickens.
[1]

Idea that:

- Bacteriophage is more specific in targeting their host bacteria (complementary receptors), while antibiotics tend to have wider host range.
- Possible emergence of antibiotic resistant bacteria due to prolonged/inappropriate use of antibiotics, but no / low possibility of bacteriophage-resistant bacteria since they naturally infect bacteria.
- Bacteriophages are harmless to chicken/human, while antibiotic may trigger allergy response.

  (Also applicable to humans since humans are consumer of the chickens.)

[Total: 14]

2017 / H2 / NYJC PRELIM / P2 Q8

1
2
3
4
5
6
7

8 Heart muscle cells and epidermal cells were extracted from Chinese hamsters. The cells were lysed and the mitochondria and cytosol were isolated. The mitochondria and cytosol were then mixed and re-suspended in a culture of essential nutrients. This suspension system was used to study the process of cellular respiration.

At time 0, glucose was added to the system. At Time X, Digitonin, a detergent which disrupts membranes was introduced to the suspension system. A probe was used to measure the concentrations of ATP as well as the pH level in the mitochondria.

The experimental results are recorded in the graphs shown. **Fig. 8.1** shows the rate of ATP production for heart muscle cells and epidermal cells. **Fig. 8.2** shows the pH level of the mitochondria in both heart muscle and epidermal cells.

Fig. 8.1

Fig. 8.2

Fig. 6A

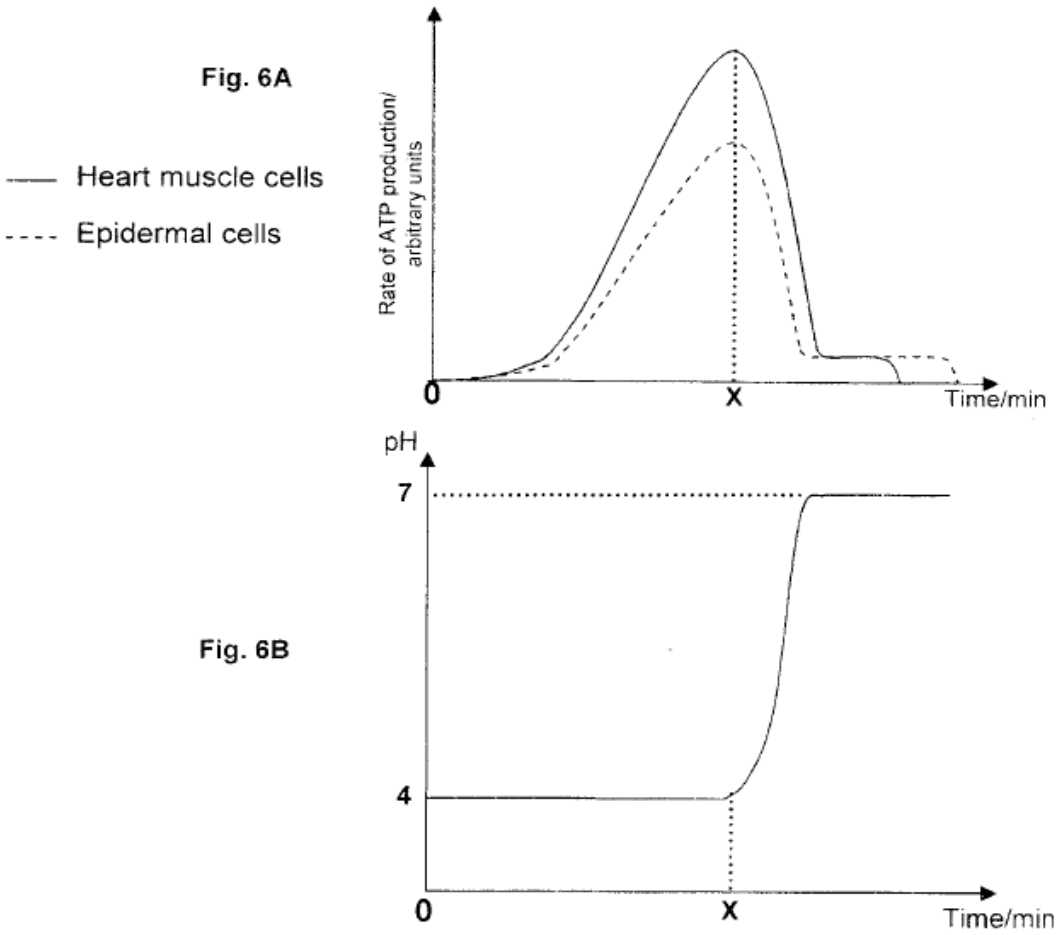


Fig. 6B

(a) Account for the difference in the level of ATP production in both tissues after glucose was added.

Heart cells produce a higher level of ATP as it contains more mitochondria than epidermal cells;

[1]

(b) With reference to **Fig. 8.1**, explain the changes in ATP production over time for the heart muscle cell suspension.

2 marks max for the first 3 marking points

High energy electrons from reduced coenzymes are passed down a series of electron carriers on the ETC, each with an energy level lower than the one preceding it;

Energy from the flow of electrons is used to actively pump protons from matrix to intermembrane space, through conformational change of proteins in ETC;

Rate of ATP production increases with time before addition of X due to protons diffuse down the electrochemical proton gradient back into mitochondrial matrix through ATP synthase/ stalked particles, synthesizing ATP from ADP and Pi;

Ref to effect resulting from the membrane damage e.g. no ETC / ATP synthase / electrochemical gradient etc

Initial lag in ATP production because glycolysis is occurring to produce 2 ATP per glucose by substrate level phosphorylation;

OR

Rate of ATP production levels off because glycolysis can still occur and decreases to zero when glucose is used up;

7 *Streptococcus pyogenes* bacteria causes a range of diseases including skin infections and respiratory illnesses. Treatment of the diseases is carried out using antibiotics such as penicillin and erythromycin.

[3]

In 1988, a nation-wide movement to reduce the use of the antibiotic erythromycin to treat patients infected with *S. pyogenes* was started in Finland.

Fig. 7.1 shows the number of doses of erythromycin used per thousand people per month, over a period of eleven years from 1984 to 1994. The figure also shows the percentage of infections each year caused by erythromycin-resistant strains of *S. pyogenes*.

(c) With reference to **Fig. 8.2**, state which region of the mitochondrion the pH probe was measuring. Explain your conclusion.

Intermembrane space;

Because it is the only part that contains H+ ions;

[2]

(d) Suggest why cytosol was used to re-suspend the mitochondria.

Enzymes involved in glycolysis are present in the cytosol;

[1]

(e) From your biological knowledge, explain the adaptation of the double membrane for its role in the production of energy.

Membrane is impermeable to protons, creating electrochemical proton gradient across the inner mitochondrial membrane;

Highly folded inner membrane increase surface area for stalked particles containing ATP synthase and electron carriers to be embedded;

Compartmentalisation so that reactions can occur in different locations (ref. to provision of optimal conditions for enzymes such as that for Krebs' Cycle to work);

[2]

[Total: 9]

2017 / H2 / RI PRELIM / P2 Q7

Fig. 7.1

(a) (i) Describe two structural features that are typical of bacteria, including *S. pyogenes*. [2]

	Characteristic	Description
1	Cell wall	Prevents osmotic lysis of cell protoplast and confers rigidity and shape to cells, composed of peptidoglycan
2	Chromosome (location)	Found within nucleoid region/ no true nucleus
3	Chromosome (nature)	A single, circular, double helix DNA, no introns
4	DNA-associated proteins	Proteins anchoring loop domains present
5	Organelles	No membrane-bound organelles
6	Plasmids	Extra-chromosomal DNA that replicates autonomously;
7	Ribosomes	70S (vs 80S in eukaryotes)
8	Appendages (any one) a. Fimbriae b. Pili c. Flagella	Fimbriae - Attachment to surfaces and to other bacteria/organisms Pili - Mediates DNA transfer during conjugation (sex pilus)/ Motility by retraction Flagella - Swimming movement, propulsion Accept if describe structure or purpose.
9	Capsules or Slime layers	Capsules - protection against phagocytic engulfment / attachment to surfaces/ contains water to prevent desiccation Slime layers - attachment to surfaces / to form biofilm Accept if describe structure or purpose.

(ii) Explain the advantages to scientists of giving the bacterium *Streptococcus pyogenes* a binomial Latin name. [2]

1. Universal name to avoid ambiguity;
2. Once an organism can be identified, it can be organised into groups/ taxons according to shared characteristics;

(b) (i) With reference to Fig. 7.1, describe the trend in the use of erythromycin between 1984 and 1994. [3]

1. Increase – 1.6 doses per thousand people per month in 1984 to 2.2 doses per thousand people per month in 1987/ to 3.0 doses per thousand people per month in 1988;

2. Maximum - 3.0 doses per thousand people per month in 1988;
3. Decrease slightly - to 2.25 doses per thousand people per month in 1990/ to 2.3 doses per thousand people per month in 1991;

A: Decrease dramatically – from 2.3 doses per thousand people per month in 1991 to 1.25 doses per thousand people per month in 1992;

4. Slight variation/ almost plateau – 1.2 to 1.3 doses per thousand people per month from 1992 to 1994;

(ii) Apart from mutation, suggest how the erythromycin-resistant *S. pyogenes* may have originated in Finland in 1986. [1]

1. erythromycin-resistant bacteria brought in from outside Finland;
2. conjugation where there is transfer of plasmid with erythromycin-resistant gene;
3. transformation where there is uptake of foreign DNA coding for erythromycin-resistance;

Note: If transduction mentioned, detailed elaboration required – phage, resistant gene, from another bacteria

(iii) Explain why the percentage of erythromycin-resistant bacterial infections increased between 1986 and 1993. [2]

1. Use of erythromycin at as a ***selection pressures****;
2. Antibiotic resistant bacteria have a selective advantage and they are selected for;
3. increasing frequency of favourable alleles/ and will survive, reproduce and pass on their alleles to the next generation during microevolution;
4. Percentage of infections caused by erythromycin-resistant strains increased from 2.5% in 1986 to 1.9% in 1993;
5. There is a time lag between erythromycin used falling and percentage of resistant infections falling;

(iv) Suggest why the percentage of erythromycin-resistant bacterial infections fell between 1993 and 1994. [2]

1. fewer doses of erythromycin used;
2. now less of a selection pressure for resistant strains therefore non-resistant survive/ have a selective advantage/ outcompete resistant strains;
3. a) non-resistant strains survive better because resources not used for resistance against antibiotics (eg: inactivating antibiotics)

   OR

   b) non-resistant strains survive better because resources may be diverted to growth/reproduction;

(c) In 2014, the World Health Organisation highlighted the prevalence of antibiotic resistance in bacteria as a global health threat. This prompted the urgent development of alternative methods to the use of antibiotics to treat bacterial infections.

Phage therapy, which is the use of bacterial viruses to defend against pathogenic bacteria is a strategy to address this issue.

Suggest an advantage of using phage therapy. [1]

1. Phage infects specific bacterial strains so there is little impact on useful bacteria in host, unlike antibiotics;
2. Phages consist mostly of nucleic acids and proteins, they are generally non-toxic/less side effects;
3. Only a small quantity of phage is needed as phages can replicate once it infects the bacteria to produce more viruses;
4. OR
5. Replicate at site of infection and thus available where they are most needed (unlike antibiotics that move through the whole body)
6. As bacterial host cell evolve to evade phage infection, phage may co-evolve. If the new phages are isolated, they can be used for phage therapy;
7. Phages are easily discovered e.g. sewage and waste materials that contain high bacterial

concentrations;

8. Some antibiotics are bacteriostatic and may not kill bacteria, leading to development of antibiotic resistance whereas bacteria infected by lytic phages will definitely die.

[Total: 15]

2017 / H2 / RVHS PRELIM / P2 Q5

9 Fig. 5.1 shows the structure of a T4 virus.

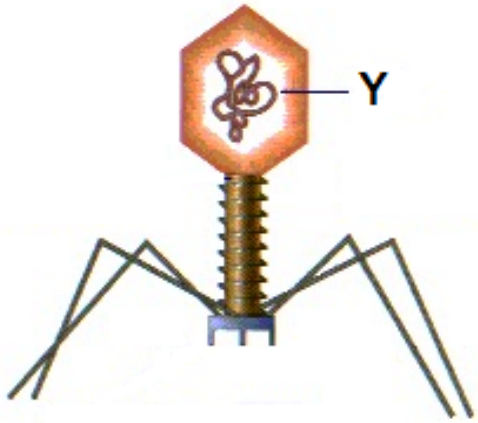


Fig. 5.1

(a) Identify structure **Y**. [1]

Double-stranded deoxyribonucleic acid;;

The T4 virus cannot reproduce by itself and relies upon a host cell for reproduction.

(b) State specifically why T4 viruses rely on host cells for their reproduction. [2]

1. **lacks a named enzyme (e.g. RNA polymerase / DNA polymerase);;**
2. **lacks a named organelle (e.g. golgi apparatus for protein modification / RER for protein synthesis);;**
3. **lacks a named molecule for protein synthesis / DNA replication;;**
4. **lacks a named energy resources, e.g. ATP;;**

T4 viruses use bacteria as its host. Fig. 5.2 shows the results of an experiment in which T4 viruses were added to a culture of bacteria. Samples of the culture were then taken at intervals to determine the number of free T4 viruses present.

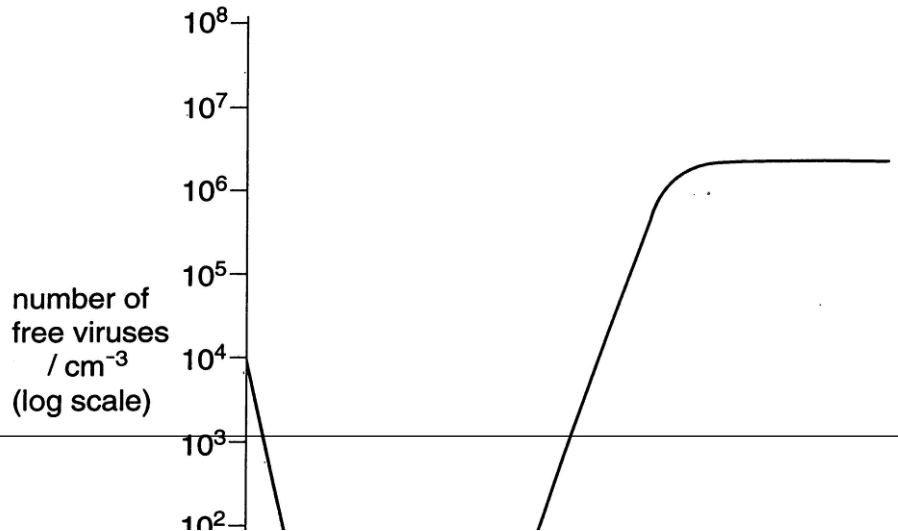


Fig. 5.2

(c) With reference to Fig. 5.2, describe and explain the changes in number of free T4 viruses

(i) in the first 10 minutes; [2]

1. **number of free viruses decreases from 10^4 to 10^1 cm^{-3};;**
2. **Due to attachment of viruses on the *E. coli*;;**

Reject: viruses enter host cell

(ii) between 30 and 60 minutes. [3]

1. **number of free viruses increases from 10^1 to 10^6 cm^{-3};;**
2. **due to lysis of host cell to release viruses;;**
3. **(increase in number of viruses from 10^4 to 10^6) due to multiplication of viruses;;**

A scientist carried out an investigation using T4 virus and two strains of bacteria: **B^+** cells which can grow in media without lysine and **B^-** cells which only grow when supplied with lysine. The procedure is shown in Fig. 5.3.

T4 are mixed with **B^+** cells

↓

T4 are isolated from the culture and added to **B^-** cells

↓

B^- cells are plated on medium lacking lysine

↓

Growth observed on medium

Fig. 5.3

(d) **(i)** Explain the observations made by the scientist. [3]

			1. (Generalised) transduction; **2. T4 infects B^+ cell;** **3. Fragment of B^+ DNA confers ability to produce lysine;** **4. are accidentally packaged into phage capsid;** **5. Upon release from B^+ cell, transducing phage infects new B^- cell;** **6. B^+ DNA incorporated into B^- DNA (via homologous exchange);** **Penalise 1 mark for lack of contextualisation**	
		(ii)	Suggest **one** other potential benefit of the process mentioned in **(d)(i)** for the recipient bacteria.	[1]
			1. Develop antibiotic resistance/ xenobiotic (chemical) resistance;; **2. Ability to utilise a new metabolite;;**	
				[Total: 12]

2017 / H2 / SAJC PRELIM / P2 Q4

QUESTION 10

Researchers have identified a gene that gives bacteria resistance to a type of antibiotics called polymyxins. Despite being discovered around 60 years ago, polymyxins maintained their effectiveness as antibiotics as they were seldom used due to concerns about their toxicity.

In recent years, rampant use of common antibiotics (e.g. penicillin) has led to the emergence of bacterial strains which are resistant to these antibiotics. This has become more and more of a global concern. Polymyxins are now a last line of defense against bacteria because of its previous lack of use.

(a) With reference to the reproductive cycle of bacteriophages, suggest how bacteriophage infections may lead to a spread of antibiotic resistance between bacterial populations.

……..……..…[3]

1 During generalised / specialised <u>transduction</u>, **host/bacteria DNA** can be incorporated into the phage capsid **randomly (for generalised transduction)**/**occasionally**/**by mistake** during viral assembly;
2 The resulting transducing phages infect other bacteria and newly infected cell acquires the donor bacterial DNA
3 Genetic recombination occurs and **expression** of antibiotic resistance genes result in phenotype of antibiotic resistance–

Bacteria reproduce by the process of binary fission.

(b) Explain the significance of binary fission in bacteria

……..……..……[2]

1 Ref. <u>asexual reproduction</u> for unicellular organism
2 Ensuring that offspring are <u>genetically identical</u> to the parent / Desirable alleles/traits are passed down
3 Rapid increase in cell numbers (under favourable conditions)

[Any 2]

The process of binary fission involves semi-conservative DNA replication.

(c) State two differences in the formation of the leading and lagging strands during DNA replication.

……[2]

1 Presence of DNA ligase in lagging strand to ligate Okazaki fragments;
2 Presence of Okazaki fragments in lagging strand but none in leading strand;
3 Presence of more than 1 (RNA) primer/primase in lagging strand;
(**REJECT**: "no primer needed in leading strand". This is incorrect!)
4 Strands are synthesized in opposite directions;
5 Leading strand is synthesized continuously vs lagging strand is synthesized discontinuously in the form of okazaki fragments

[Any 2]

Bacteria rely on sugar sources e.g. lactose for survival.

(d) Describe the consequence of mutating the *lacI* gene of the bacterial lac operon, on usage of lactose.

……[5]

1 lac repressor has a change in 3D conformation at the **DNA-binding domain** / **allosteric site** so that allolactose / inducer binds tightly
2 lac repressor is inactive and is no longer able to bind to the operator (lacO) ;
3 RNA polymerase can constitutively access and transcribe the structural genes / lacZ , lacY and lacA;
4 β-galactosidase, lac permease and lactose transacetylase / (inducible) enzymes to utilize lactose are **constitutively** synthesised ;
5 Bacteria can utilize lactose even in the absence of lactose;

OR

1 lac repressor has a change in 3D conformation at the **DNA-binding domain** / **allosteric site** so that allolactose / inducer cannot bind
2 lac repressor (super repressor) binds tightly/continuously to the operator (lacO) ;
3 RNA polymerase cannot access and transcribe the structural genes / lacZ , lacY and lacA;
4 β-galactosidase, lac permease and lactose transacetylase / (inducible) enzymes to utilize lactose are **not** synthesised ;
5 Bacteria cannot utilize lactose even in the presence of lactose;

[Q4: 12 marks]

2017 / H2 / SAJC PRELIM / P2 Q5

11 Operons in bacteria allow them to regulate their gene expression in response to changes in the environmental conditions.

In order to investigate the function of the regulatory and structural genes of *lac* operon, loss-of-function mutation was induced in the sequences of various genes. The different effects of the mutation on the expression of *lac* genes are shown in Table 5.1.

Table 5.1

Region of DNA sequence in which gene mutation occurs	Allolactose absent		Allolactose present	
	β-galactosidase	transacetylase	β-galactosidase	transacetylase
A	+	+	+	+
B	–	–	–	–
C	–	–	–	+

D	–	–	+	–

(+) indicates the synthesis of functional enzyme
(–) indicates no synthesis of functional enzyme

(i) Identify regions **A** and **D**. [2]

A: *lacI*/ *lacI* promoter/ operator
D: *lacA*

(ii) Outline the effect of the mutation of region **A** on the expression of *lac* genes. [2]

1. **The lac repressor is not synthesized/ non-functional,**
2. **therefore it is unable to bind to the operator.**

OR

1. **Operator is mutated**
2. **lac repressor cannot bind to the operator.**

3. **Thus, regardless if the inducer allolactose is present or absent,**
4. **RNA polymerase is able to bind to the promoter to transcribe the genes of the *lac* operon.**

Mammals respond to changes in the environmental conditions using different mechanisms. For instance, blood glucose concentration can be regulated by hormones such as insulin and glucagon.

Fig. 5.1 shows the modification of preproinsulin to form insulin in organelles **X** and **Y**.

Fig. 5.1

With reference to Fig. 5.1, outline what happens in organelles **X** and **Y**. [2]

X: Rough endoplasmic reticulum

1. **The signal peptide of preproinsulin is cleaved in the rough endoplasmic reticulum to form proinsulin.**

2. **Two disulfide bonds were formed between the A and B chains.**

Y: Golgi apparatus

3. **The C-chain is cleaved/ hydrolyzed by (proteolytic) enzymes to form a functional insulin.**

Fig. 5.2 shows the effect of glucose on a pancreatic cell.

Fig. 5.2

With reference to Fig. 5.2, outline how the pancreatic cell responds to elevated blood glucose levels. [3]

1. **Glucose enters the β-cells via facilitated diffusion through GLUT2.**
2. **Glucose is broken down into ATP.**
3. **The binding of ATP to ATP (sensitive)-K^+ channel closes the ATP-K^+ channel, causing the depolarization of the plasma membrane.**
4. **This triggers the opening of Ca^{2+} channels, thus resulting in the influx of Ca^{2+} into the cell.**
5. **triggers the fusion of insulin-containing secretory vesicles with the plasma membrane**
6. **to release insulin into the bloodstream.**

Mammalian hormones can be synthesized artificially using bacterial cells.

Suggest **one** problem associated with expressing mammalian genes in bacterial cells. [1]

ANY ONE:

1. **Introns are present as bacterial cells cannot carry out RNA splicing.**
2. **Eukaryotic promoter sequences / control elements may not be recognized by the bacteria, gene not expressed.**

Compare the advantages of a mammalian response to changes in blood glucose concentration with that of a bacterial response to changes in supply of lactose. [2]

Similarities

1. **Both allow the organism to utilise the increase in the supply of carbohydrates (glucose/ lactose).**

Differences

		Mammalian response	Bacterial response
1.	Rate of response	Respond faster	Respond slower
2.	Synthesis of proteins	Hormones are synthesized and stored.	Enzymes are synthesized when required
3.	Regulation of carbohydrate	Able to regulate glucose supply	Unable to regulate glucose supply

supply

[Total: 12]

2017 / H2 / VJC PRELIM / P2 Q3

12 (a) (i) Identify substrates **X** and **Y**.[2]

X: glucose;

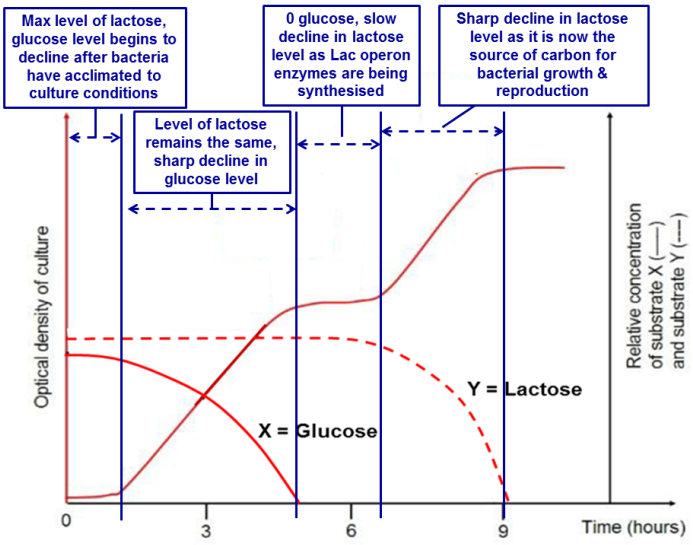
Y: lactose;

(ii) Using your knowledge of gene expression in bacteria, explain how Fig. 3 supported their conclusion that the *Lac* operon is under dual control. [4]

- Evidence #1 – first growth phase: When glucose and lactose are both present, glucose is used preferentially (A! first/ preferred respiratory substrate) for bacteria to grow and reproduce;;
- Lac operon is under negative control and the gene coding for beta-galactosidase that breaks down lactose into glucose and galactose is not expressed;;
- Evidence #2 – second growth phase: when glucose is depleted, lac operon is active and under positive control, so expression of the gene for beta-galactosidase is upregulated;;
- Evidence #3 – Lag phase: bacterial growth levels / plateaus out: time needed for activation of CAP by cAMP when adenylyl cyclase inhibition is removed after glucose is depleted and cAMP levels increase/ time needed for the expression of lac operon;;

(b) On Fig. 3, draw separate graphs to show the change in the concentration of the two substrates over time. Label your graphs. [2]

Answer:



Each curve for glucose and for lactose must show:

- Decreasing trend but with glucose being used first @½ m
- Shape of curve to consist of plateau and linear segments @½ m
- A! decreasing trend for glucose from time 0. (FYI – During the lag phase, bacteria are adapting to growth conditions, so that individual bacteria are maturing and not yet able to undergo binary fission. During the lag phase, synthesis of RNA, enzymes and other molecules occurs. As the cells are metabolising, there is some usage of glucose.)

(c) Eukaryotes are structurally different from prokaryotes and hence exhibit differences in their control of gene expression.

Explain two such differences. [4]

Any two below (note: from perspective of eukaryotic genes):

- Chromatin modelling – acetylation/deacetylation Or methylation/demethylation of CpGs in eukaryotic promoters;; to compact chromatin by wrapping high mw DNA/ large eukaryotic

genome around histones to fit into space of nucleus;;

- Post-transcriptional – 5’ capping and polyA tail addition;; for protection from exonucleases / facilitate transport out of nucleus through nuclear pores to the cytoplasm;;
- Post-transcriptional – alternative splicing of pre-mRNA occurs;; to produce more than 1 type of mature mRNA/ protein product from one gene / to generate more types / diversity of proteins than no. of genes in genome (to perform all the functions necessary for cell to survive/ in response to different stimuli);;
- Translational - through addition of long polyA tail;; to maintain stability of mature mRNA in the cytoplasm as templates for translation of more protein;;
- Post-translational – protein modification;; to activate/ inactivate proteins in response to appropriate signals/ control activity of synthesised proteins;;
- A! Transcriptional – In eukaryotes, one promoter controls the expression of one gene (instead of several functionally related genes);; because eukaryotic genes are not organised into operons (explanation);;
- A! presence of many control elements distal from gene;; allowing for combinatorial control of expression;;