2016 H2 Biology P2 9744/03

1 (a) Describe the natural functions of these restriction enzymes. [2]

1. Used as a defense mechanism by bacteria against bacteriophage by cutting up foreign DNA, hence restricts multiplication of viruses;
2. Enzyme that recognizes and bind a specific 4-6 base pair DNA sequence called a ***restriction site**** as its active site is complementary to the DNA sequence;
3. Enzyme cuts/breaks ***phosphodiester bonds**** on specific positions on both DNA strands;

(b) (ii) Use the information in Table 1.1 to compare the effect of pH on immobilised P1 nuclease and free P1 nuclease. [4]

1. Compare peak + quote data
   The optimum pH for immobilized P1 nuclease was higher at pH 6.0 whereas free P1 nuclease peaked at pH 5.5;
   or
   The maximum nuclease activity for immobilized P1 nuclease was higher at 68 arbitrary units(a.u.), whereas maximum nuclease activity for free P1 nuclease was 65 arbitrary units.
2. Immobilised P1 nuclease activity was lower than free P1 nuclease from pH 5 to pH5.5. + quote data from either pH
   e.g. immoblised P1 nuclease activity was 49 a.u. whereas free P1 nuclease was 56 a.u. at pH 5.0, or immoblised p1 nuclease activity was 7 a.u. lower than free P1 nuclease at pH 5.0
3. Immobilised P1 nuclease activity was higher than free P1 nuclease from pH6.0 to pH7.0. + quote data from any 1 pH
   e.g. immoblised P1 nuclease activity was 68 a.u. whereas free P1 nuclease was 48 a.u. at pH 6.0, or immoblised p1 nuclease activity was 20 a.u. higher than free P1 nuclease at pH 6.0);
4. Both had similar trend of increasing in activity from pH4.5 to pH5.5 then decreasing from pH6.0 to pH7.0 + quote data

(ii) With reference to your knowledge of enzyme structure and the information in **(b),** explain the higher activity of immobilised P1 nuclease, compared to free P1 nuclease, at temperatures above 30 °C, as shown in Table 1.2. **[3]**

1. At higher temperatures, intramolecular vibrations increase, which results in the breaking of bonds that determine the conformation of the enzyme in the free P1 nuclease.
2. Resulting in the denaturation of the enzyme due to a change in conformation of the enzyme active site, decreasing the rate of enzyme activity of free P1 nuclease
3. Binding of P1 nuclease to matrix, stabilize the enzyme's structure at higher temperatures, reducing intramolecular vibrations, prevents breaking of bonds, and retaining the conformation of enzyme active site.
4. Activity of free P1 nuclease starts to drop at 60°C which is lower than that of 70°C in immobolised P1 nuclease.

(ii) Suggest why P1 nuclease was needed in the production of this particular recombinant DNA molecule. [3]

1. P1 nuclease was needed to break down the different sticky ends produced by the digestion of HindIII and EcoRI.
2. As it is able to break down single-stranded DNA, leaving the double stranded DNA fragment intact.

3. Scientist will then be able to use the double stranded DNA fragment to synthesize complementary stick ends/add ligase to join the desired DNA fragments directly to form a recombinant DNA molecule.

2 (a) From your knowledge of collagen structure, explain why the length of any exon is divisible by 9. [3]

1. Collagen has a repeating amino acid sequence of glycine-X-Y/three amino acids;
2. Where each amino acid is deterimined by a codon of three nucleotides on the mRNA,
3. Thus, three of such codons will be 9 nucleotides in the exon.

3 (a) The genetic disease X-linked SCID (severe combined immunodeficiency) can be treated by bone marrow transplants. This process can be carried out very early in a child's life.

(i) The bone marrow contains blood stem cells that are described as being multipotent. State what is meant by multipotent. [1]

1. Multipotent stem cell has ability to ***divide* and differentiate**** to form limited range of cell types / differentiate into red and white blood cells.;

(ii) Describe the characteristics that identify all stem cells. [2]

1. A stem cell is an ***undifferentiated/unspecialized**** cell capable of undergoing ***proliferation* and self-renewal****;
2. and retains potential to ***differentiate**** to produce specialized cells upon receiving appropriate ***molecular signals****;

(c) (i) Suggest how the mitochondrial genome can code for about 40 genes when it is only four times the size of the SCID gene. [2]

1. As mitochondrial genome are similar to prokaryotic genome, they are much smaller, as it has lesser non-coding sequences compared to genes in the chromosomes.
2. SCID genes are large due to ***introns**** which are sequences that are spliced out during post-transcriptional modifications.

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4 **Main theory**

- Effectiveness of active compounds in garlic solution against *Staphylococcus* can be determined by placing garlic solutions of different concentrations onto wells on ***prepared 100 mm diameter agar plates, with a lawn of Staphylococcus sp.*.***
- Garlic solution will diffuse through agar and inhibit growth of bacteria.

Measurable quantity

- This results in a clear zone around the well that has no bacteria growth. This is known as zone of inhibition and its diameter is a measure of effectiveness of garlic solution against bacteria.

Trend

- The higher the concentration of garlic, the more it will diffuse across agar, hence a zone of inhibition with a larger diameter due to more bacteria dying.
- Most effective concentration of garlic solution is lowest concentration of garlic solution that gives largest zone of inhibition. Further increase in concentration of garlic solution does not increase diameter anymore.

Independent variable

- ***20% garlic solution**** diluted at 4 concentrations using syringes and sterile ***distilled water**** should be prepared first, under aseptic techniques:

Concentration of garlic solution (%)	Volume of stock garlic solution (ml)	Volume of ***distilled water**** (ml)
20	10.0	0.0
16	8.0	2.0
12	6.0	4.0
8	4.0	6.0
4	2.0	8.0

Dependent variable

- Growth of bacteria, as indicated by diameter of zone of clearance (mm) measured using a mm ruler.

Controlled variables

1) Volume of garlic solution poured into wells on agar plate
   - (how) using same cork borer eg: 10 mm diameter, puncture 6 wells of same size on given Petri dish with agar
   - (why) wells need to be same size so that constant volume of garlic solution will be placed in the wells as amount of active compounds in garlic solution affects bacterial growth
2) Temperature where bacteria is incubated
   - (how) incubator set at 37°C for 12 hours
   - (why) temperature needs to be kept constant as bacteria growth is affected by temperature

Replicates

- (how) 3 replicates are the 3 different petri dishes, each with 5 wells with different concentrations of garlic solution
- (why) check for anomalous results

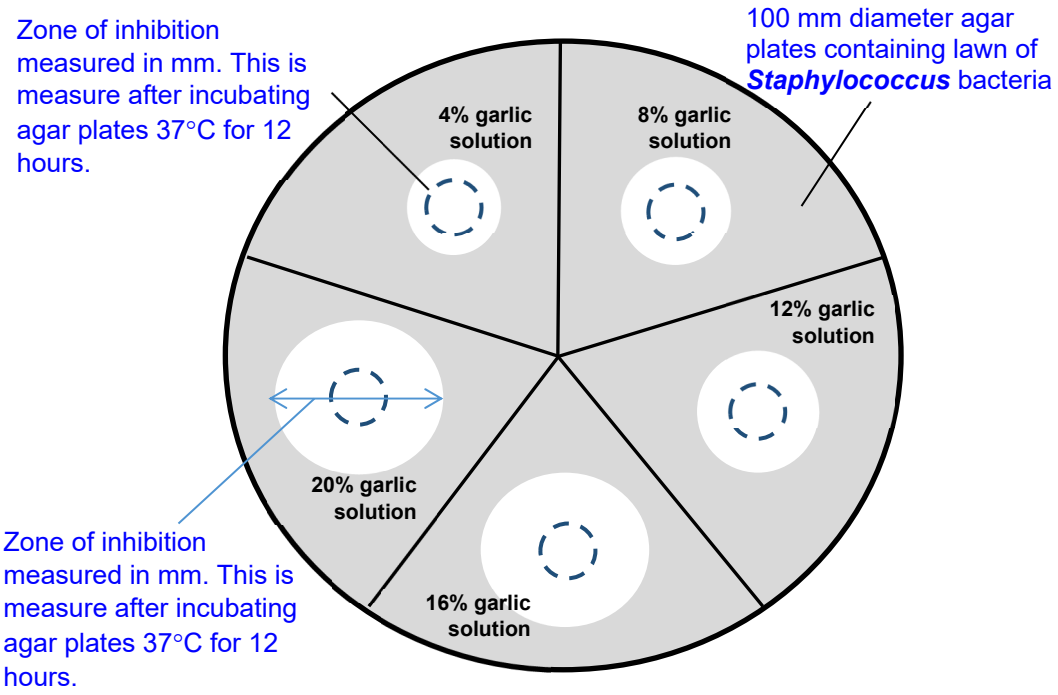
Repeats

- (how) repeat entire experiment 2 more times
- (why) check for reproducibility

Negative control

- (what) set up control experiment by filling up well with sterile distilled water on bacteria lawn. All other variables and procedures should remain constant.
- (why) control shows no clear zone, where bacteria growth is not inhibited. Bacterial growth in other wells is due to presence of garlic solution.

Annotated diagram

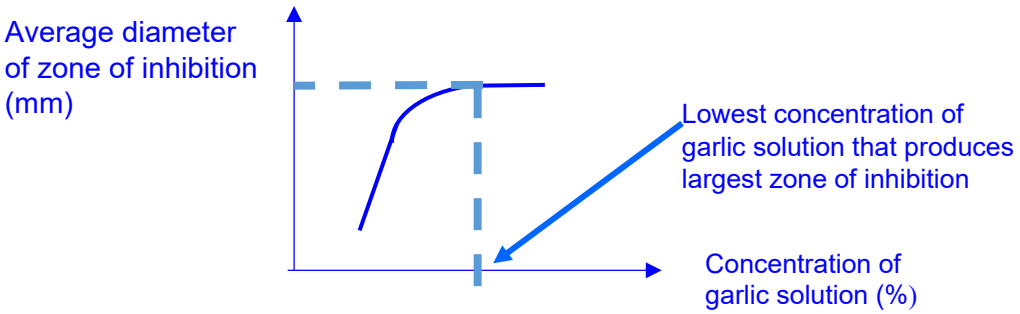


Note: In diagram, 16% and 20% garlic solutions produce zones of same diameter. That means 16% garlic solution is minimum concentration.

Fig. 1 - Set up of experiment to find lowest concentration of garlic solution that gives largest zone of inhibition

Results

Concentration of garlic solution (%)	Diameter of zone of inhibition (mm)			Average diameter of zone of inhibition (mm)
	Replicate 1	Replicate 2	Replicate 3	
20				
16				
12				
8				
4				



Risks and precautions

Experiment needs to be carried out under sterile microbiological techniques to prevent contaminating bacteria from growing on agar plates. Before any work is started, bench must be ***wiped with disinfectant (sterilizing) solution and paper towels.**

Additional precautions (name 1):
e.g.: cork borer need to be sterilized using autoclave
e.g.: when placing garlic solutions into wells on agar plate, pipette garlic solution using micropipette tips that have been sterilized using autoclave. It is important to work near a lighted up Bunsen burner
e.g.: water need to be sterilized using autoclave
2. As bacteria may be harmful, gloves must be used when handling agar plates. Used materials including agar plates need to be properly disposed of by sterilizing using autoclave.

Improvement

Lowest concentration of garlic solution – experiment can be further refined to find exact concentration of garlic solution. Repeat experiment under same conditions, use smaller intervals
e.g.: If minimal concentration is 16%, repeat with 15.0,15.5%, 16.5%, 17.0%, 17.5% garlic solution.

5 (a) Explain what is meant by restriction fragment length polymorphism (RFLP). [5]

1. Restriction Fragment Length Polymorphism (RFLP) refers to the unique banding pattern among individuals when the genomic DNA are digested by restriction enzymes separated by gel electrophoresis;
2. e.g 1. single nucleotide polymorphisms (SNPs, pronounced 'snips') / differences at a single base-pair in DNA sequences in homologous regions;
3. due to mutations;
4. can result in variations in number and/or location of restriction sites**;**
5. Due to e.g 2. variations in the number of tandem repeats**;**
6. e.g. 1 or 2 produce fragments of different length among individuals called restriction fragment length polymorphisms (RFLPs);
7. Upon visualization and analysis, a unique banding pattern is produced for each individual;

(b) Outline the process of nucleic acid hybridisation. [10]

1. To detect the RFLP pattern of a gene, the DNA sample is digested with restriction enzyme and;
2. Then the digested DNA samples are separated using gel electrophoresis;
3. ds DNA is denatured / made single-stranded and by alkaline / NaOH solution;
4. and transferred to a nitrocellulose membrane;
5. at exactly the same position as they were in the gel;
6. The nitrocellulose membrane incubated with a ***radioactive single stranded DNA probe*****,** that is ***complementary in sequence**** to part of the target sequence / gene;
7. DNA fragments containing this part of the target sequence will hybridise to the probe **by *complementary base-pairing**;**

8. After hybridisation, membrane is washed to remove any excess unhybridised probes.
9. Using ***autoradiography/ by placing X-ray film**** over the membrane, the banding pattern can be visualised;
10. The radioactivity of the bound probes exposes the film to form an image corresponding to the bands that have base-paired to the probe;

(c) Explain how RFLP analysis facilitates the process of DNA fingerprinting. [5]
[not in syllabus but good to know]

1. When the genomes from 2 individuals A and B, are cut using the same restriction enzyme (e.g. BamHI);
2. If the 2 individuals differ in the number of tandem repeats (of the sequence ATTAC) at a particular locus on particular chromosome;
3. The individual with the larger number of tandem repeats present, (e.g. individual B) generates a longer restriction fragment which travels a shorter distance down the gel during electrophoresis;
4. while the individual with fewer number of tandem repeats present, (e.g. individual A) generates a shorter restriction fragment which travels a longer distance down the gel during electrophoresis;
5. The 2 different-sized fragments are detected using the same probe which has a sequence complementary to segments of both fragments e.g. ATTAC.
6. Will result in fragments of different lengths being produced;
7. Can be discriminating enough to generate a DNA fingerprint / unique genetic profile for each individual.