BIOTECHNOLOGY

CLASS XII

There will be two papers in the subject

Paper I: *Theory:* *3 hours ... 70 marks*

Paper II*: Practical:* *3 hours ... 15 marks*

Project Work ... 10 marks

Practical File ... 5 marks

PAPER I –THEORY- 70 Marks

*There will be **one** paper of **three** hours duration divided into **two** parts.*

Part 1 (20 marks) *will consist of compulsory short answer questions, testing knowledge, application and skills relating to elementary/fundamental aspects of the entire syllabus.*

Part 2 (50 marks) *will consist of **eight** questions out of which the candidates will be required to answer **five** questions. Each question in this part shall carry 10 marks.*

1. Molecular Biology

(a) Biomolecules: Introduction to biomolecules-definition and types. Carbohydrates, proteins, lipids, vitamins and enzymes – their structure and properties.

Structure, functions of carbohydrates.

Sugars and derivatives; and classification of some important mono, di and polysaccharides - Glucose, fructose, glycogen, cellulose, chitin and peptidoglycon. Physical and chemical properties of sugars.

Structure, functions and classification of proteins – building blocks of proteins, the amino acids. Chemical structure, types (acidic, basic and neutral); physical and chemical properties of amino acids. Different methods employed in determining the amino acid sequence in proteins: 3D - structure of proteins. Different types of proteins - primary, secondary, tertiary quarternary.

Vitamins: Definition of fat soluble and water soluble vitamins; co-enzymes: definition and examples.

Enzymes: Structure and functions of enzymes: chemical nature of enzymes; characteristics and properties of enzymes. An understanding of enzyme activity; mechanism of enzyme action - Lock and key model and induced fit hypothesis; factors affecting enzyme activity.

Structure and functions of lipids – building blocks of lipids, their structures, types and chemical properties.

Optical activity of biomolecules.

Concept of supramolecular assembly.

(b) Nucleic acids: an understanding of nucleic acids, their importance in biotechnological work, biochemical structure and capacity to replicate.

DNA – physical and chemical structure; definition, double helical model of DNA, (Watson and Crick's); Nucleotide and nucleoside; Chargaff's Law method of replication of DNA, various replicative enzymes in both procaryotic and eucaryotic organisms, example topoisomerases, helicase, SSBs polymerases, primases, ligases. Concept of semi conservative and semi-discontinuous replication, leading and lagging strands, okazaki fragments.

RNA – definition, various types of RNAs such as mRNA, tRNA (Clover leaf model), their structure and functions.

(c) Protein Synthesis: synthesis of different RNAs, and the complete mechanism of polypeptide chain formation. Different metabolic diseases which occur due to a change in the DNA structure.

From gene to protein: Transcription - DNA to RNA, various enzymes involved eg RNA polymerases, amino acyl tRNA synthetase, an explanation of the complete process; post transcriptional changes- polyadenylation, 5' capping and splicing .

Genetic code – properties of genetic code, Start and Stop codons, anticodons. The translation of RNA to protein – complete mechanism of chain initiation, elongation and termination, the role of tRNA, mRNA and rRNA in protein synthesis. ***(Post translational changes not included)***

Concept of central dogma. Concept of Reverse transcription, enzyme reverse transcriptase. An understanding of one gene one enzyme hypothesis. Fine structure of gene - exon, intron.

Gene regulation – Operon concept – lac operon and trp operon.

Inborn errors of metabolism - basic concept and examples like Albinism, sickle cell anaemia. Phenyl ketonuria, alkaptonuria.

2. Genetic Engineering

(a) Innovations in Biotechnology: select examples of products already available, produced by using modern biotechnological tools.

(i) Plants: Production of Flavr Savor tomatoes; designer oil, Bt-crops.

(ii) Healthcare: Production of recombinant hepatitis-B vaccine; insulin and interferon and edible vaccines.

(iii) Animal: Dolly the cloned sheep; stem-cells research. Characteristics of stem cells and their applications.

(iv) Environmental biotechnology: oil-eating bacteria, bioremediation.

(v) Industrial biotechnology: production of industrial enzymes.

(vi) Single Cell Protein concept (SCP) – advantages of single cell protein.

(b) Introduction to gene cloning and genetic engineering: concept of cloning and vectors.

Tools of recombinant DNA technology, types of restriction endonucleases and other enzymes used in cloning: types of vectors, such as plasmids, cosmids, phages, YACs, BACs, animal and plant viruses, role of Shuttle and expression vectors in DNA manipulation, construction of a recombinant DNA molecule; steps involved in genetic engineering and gene cloning.

Techniques involved in extraction and purification of DNA from bacterial and plants cells.

A basic understanding of DNA libraries – construction and cloning of genomic and cDNA libraries.

Transfer of recombinants into host cells - basic concept of transformation, transfection, electroporation, Liposome, microinjection, biolistic and Agrobacterium induced gene transfer, T-DNA and Ti-Plasmid.

Methods of identification of recombinants- Direct selection / Insertional inactivation / Blue-white selection.

DNA probes – definition and use.

(c) Biochemical techniques: classification of techniques based on various factors.

Classification of techniques based on various factors.

Molecular weight or size: centrifugation, gel permeation, osmotic pressure.

Polarity or charge: ion exchange chromatography, electrophoresis, iso-electric focussing, hydrophobic interaction, partition chromatography, spectroscopy colorimetry, UV visible spectrophotometry, florescence spectroscopy, crystallography and mass spectrometry.

Solubility: salt precipitation and precipitation with organic solvent.

(d) Gene analysis techniques: various techniques involved in any work in recombinant DNA technology.

Low resolution mapping techniques: gel electrophoresis, northern blotting, southern blotting.

High resolution techniques: DNA sequencing- sequencing by chemical degradation, sequencing by chain termination, automated DNA sequencing. Site directed mutagenesis.

Polymerase chain reaction (PCR)– definition, principle and the technique involved, use of the enzyme taq DNA polymerase, concept of oligonucleotide primer; significance and applications of PCR.

Human Genome Project - its objectives, the countries involved, its achievements and significance.

3. Cell Culture Technology

(a) Introduction and Techniques: basic understanding of cell culture technology and its significance in biotechnology. Different materials and methods used in this technology.

Introductory History: definition of cell culture, different types of tissues and organ cultures.

Media and aseptic manipulation: definition of media; composition of media – inorganic nutrients, organic nutrients, macronutrients, micronutrients and other important supplements. Role of auxins, cytokinins in cell tissue culture. Importance of media in cell culture. Solidifying agents and pH.

Sterilisation of apparatus and instruments used in cell culture, culture rooms and transfer area. Basic organization of a tissue culture laboratory.

Preparation and cloning of cell culture along with regeneration of single cell to whole plant, basic steps in micropropagation of plants.

Role of cell and tissue culture in plant genetic manipulation – genetic variability, invitro pollination, induction of haploidy, somatic hybridisation and genetic transformation

(b) Cell culture and cellular totipotency: types of cell culture and the concept of cellular totipotency.

Cell culture: importance of single cell culture. Different methods involved in isolation of single cells from plant organs - mechanical and enzymatic methods.

Concept and types of suspension culture: batch cultures and continuous cultures. Synchronisation of suspension cultures.

Chemical methods – starvation, inhibition, mitotic arrest and plating techniques.

Cellular totipotency: definition of cellular totipotency. Concepts like cell differentiation, dedifferentiation and redifferentiation, vascular differentiation.

(c) Germplasm conservation: definition and significance of germplasm conservation and various methods involved in it.

Definition and need for germplasm conservation. Modes of conservation: in-situ conservation, ex-situ conservation; in-vivo and in-vitro conservation; Advantages and disadvantages of in-situ and ex-situ conservation. Materials used for conservation. Principles involved in freeze preservation. Various types of freeze preservation.

(d) Applications of cell culture technology: different fields in which cell culture technology is used and the ways it is used. Application in crop improvement.

Application of cell culture technology in plant breeding: haploid production – an understanding of haploid production and in vivo techniques employed to induce haploid production such as gynogenesis, androgenesis, genome elimination by distant hybridisation and semigamy, chemical treatment, temperature shocks and irradiation effects.

Triploid production: understanding and need for triploid production. Application of triploids in plant improvement. Seedless crops.

In vitro pollination: concept, and application of in vitro pollination.

Zygotic embryo culture concept and applications.

Concept of somatic hybridisation and cybridisation, protoplast fusion, genetic transformation and their applications in plant improvement.

The scope biotechnology offers in developing favourable traits in crops, like pest resistance, drought resistance, salinity resistance. Production of Biodegradable plastic, synthetic seeds and virus free crops.

4. Bio-informatics

(a) Introduction: an introduction to computers, both hardware and software aspects. Global biological data bases.

Introduction to computer software and hardware - RAM and ROM, Microprocessor. Definition, significance and application of bio-informatics. Enormity of data generated

by biological systems; managing the data using tools provided by Information Technology.

An introduction to global bio-informatics databases (nucleotide and protein databases). Information sources such as EMBL, NCBI GDB, MGD.

Data retrieval tools- ENTREZ, BLAST, Taxonomy Browser, FASTA.

Genomics: basic understanding of genome, types of genome –prokaryotic and eukaryotic, criteria for selecting an organism for sequencing. Various theoretical aspects of searching genes using the computer.

Definition of genomics. Types of genomics-structural and functional. Basic criteria in selecting the organism for its genome sequencing. Searching for genes using computers. All the theoretical aspects – exons, intron, promoter region, coding regions, non-coding regions, Different types of sequences – cDNA, genomic DNA, ESTs (Expressed Sequence Tags) and STSs (Sequence Tagged Sites) and the different softwares used like gene scan.

Types of sequence analysis –global, local, pair wise and multiple. A mention of different computer software and programs used in sequence analysis.

(b) Proteomics: definition and introduction.

Different softwares commercially available for structural prediction of proteins. Softwares available easily on the internet, important protein databases available for the public on the internet like PDB (Protein Data Bank), PIR (Protein Identification Resources). Use of computers in new drug development research - concept of Single Nucleotide Polymorphisms (SNPs).

Biotechnology - global and Indian scenario. Various institutes, centers and funding agencies - DBT, DST, NBTB, CCMB, which deal with biotechnology and bioinformatics in India.

PAPER II

PRACTICAL WORK – 15 marks

Candidates are required to complete the following experiments.

1. <u>Sterilization techniques</u>

   (i) *Dry Physical method – heat or radiation.*

   (ii) *Wet Physical methods – steam sterilization.*

   (iii) *Chemical Sterilization/ Surface sterilization Disinfection with 70% alcohol and Sodium hypochlorite solution /savlon/carbolic acid.*

2. <u>Preparation of buffers</u>

   This experiment should be done to make the basics clear to the students. Basic calculation for buffer preparation should be known. The approach should be to utilize easily available chemicals at reasonable costs. For this "<u>Phosphate buffer, Acetate buffer and Borate buffer</u>" are good for practice. (pH 4 - pH 9.2).

3. <u>Preparation of culture media</u>

   (i) *Bacterial culture Media - Luria Bertanii (L.B.) media - Peptone/ Tryptone, yeast extract and NaCl. (Nutrient broth / Nutrient Agar).*

   (ii) *Plant Tissue culture medium (Sugars + Coconut milk + Agar Agar).*

4. <u>Preparation of slant or nutrient plates</u>

   Luria Bertanii (L.B) media to be prepared autoclaved and cooled to 60 degrees C. To prepare nutrient plates the media is poured into pre sterilized petri-dishes under a LAF. To prepare slants the media is poured into several test tubes, plugged and kept in a tilted position until it sets.

5. <u>Growth of bacteria in culture</u>

   Growth of bacteria in nutrient broth / nutrient agar. Bacterial streaking, time related growth curve of bacterial culture to be studied.

6. Identification of gram positive and gram negative bacteria by gram staining.

   (i) Prepare a bacterial smear on a slide (ii) Stain with crystal violet stain. (iii) Rinse with water. (iv) Add a few drops of iodine solution. (v) Add few drops of 90 % ethanol (vi) Counterstain with safranin solution (vii) Observe the red and blue colonies under the microscope.

7. Isolation of proteins

   (i) Pea germinating seeds proteins should be isolated in buffers. Presence of protein to be identified by colour reaction (Biuret's Test / Xanthoproteic Test).

   (ii) Milk proteins are isolated by adding 0.4 N HCl into the milk sample. Caseins start coagulating at their isoelectric point (pH 4.6) The precipitate is filtered and weighed to quantify the protein present.

8. Determination of Blood Groups

   Students can perform this experiment on their own and work out their own blood group. Proper instructions however are to be given for 'prick' – e.g. (a) Sterilize finger with alcohol/disinfectant. (b) Use only disposable sterile needle. (c) Use the needle only once and destroy it. (d) Do not prick or use blood drop in an indiscriminatory manner.

9. Salivary amylase activity on starch

   (i) To study the action of the enzyme on starch.

   Soluble starch solution (0.5% - 1%) to be prepared. Test with iodine. Collect saliva, dilute 1: 5, add 1 ml of saliva to 10 ml of starch solution. Incubate for 15 minutes. Again test for presence of starch with iodine. Also test for the presence of reducing sugars in solution.

   (ii) To study the effect of variable temperature on the activity of the enzyme salivary amylase.

10. Separation of plant pigments by chromatography: (Paper chromatograph or TLC)

    Take any leaf. Extract chlorophyll in 80% acetone. Take a strip of paper or prepare a thin layer of silica gel on a slide. Load chlorophyll extract at one end of the paper/gel. Keep paper or gel in the rising medium in test tube or jar for about 30 minutes. The rising medium should have methanol/ acetic acid, n-butanol or benzene. The rising fluid should always be at the bottom below the point of loading of chlorophylls. After 30 minutes, three spots: yellow, bluish green and light green will be observed corresponding to carotenes, chlorophyll A & chlorophyll B.

11. Identification of Plasmid DNA and Genomic (chromosomal) DNA bands in the gels (by photographs only).

    Plasmid DNA is covalently closed circular DNA (CCC-DNA). Therefore, its molecular size is very small but the molecular weight is sufficiently high. During electrophoresis, this DNA will move faster than the genomic DNA which has low density and is linear in nature.

    Therefore, the bottom-most band, much away from the rest of the bands is Plasmid DNA whereas lagging bands represent the genomic (chromosomal) DNA fragments.

12. Isolation of DNA from plant tissues

    Take half a ripe and peeled banana into a beaker and add 50 ml of extraction fluid (1.5gm table salt +10 ml liquid detergent +90 ml distilled water). Place the beaker in a water bath set at 60 degrees C for 15 minutes. Stir gently with a glass rod. Filter 5ml of cooled content into a clean test tube and add 5ml of cold 90% ethanol. DNA molecules separate out and appear as white fibres. [DNA can also be extracted from pea seeds and soaked wheat grains]

13. Estimation of proteins by colour reaction

    Bradford's Assay is a Dye binding assay based on the differential change of colour of a dye in response to various concentrations of proteins. Bradford's assay can be performed for qualitative as well as quantitative assessment of proteins in a sample.

    Dilute 1 volume of Bradford's dye with 4 volumes of distilled water. Filter the dye through Whatman filter paper and store at room temperature in a brown glass bottle. Take different aliquots of standard Bovine Serum Albumin (BSA solution), for example (0.2, 0.4, 0.6, 0.8 and 1.0 ml) in different test tubes Make up the volume to 1ml with distilled water. To each tube add 2ml of

Bradford's dye. Extent of colour development can be made by rough estimate using + signs to show the concentration of protein in the sample. Alternatively, OD can be read using colorimeter or spectrophotometer. Take the unknown sample to be estimated and perform the experiment. Similarly read the OD and note the corresponding concentration of protein in it using the graph.

14. Estimation of DNA either by Colourimeter or Spectrophotometer.

    The principle behind estimation of DNA by colourimeter is to develop some sort of colour during reaction of DNA with some chemical or colouring agents. The developed colour will have some absorption at a particular wave-length. This absorbance can be plotted against the standard curve made with the help of standard solutions and by that the amount of DNA in a given solution can be calculated.

    In case of spectrophotometric determination of DNA, the capacity of DNA to absorb UV rays in the region of 285 nm is taken as the base for working out the amount of DNA in a given solution. Again the help of a standard curve is taken to estimate the quantity of DNA.

PROJECT WORK AND PRACTICAL FILE – 15 Marks

Project Work – 10 Marks

The Project Work is to be assessed by a Visiting Examiner appointed locally and approved by the Council.

Candidates are to creatively execute **one** project / assignment on an aspect of Biotechnology.

Teachers may assign or students may choose any one project of their choice. The report should be kept simple, but neat and elegant. No extra credit shall be given for type-written material/decorative cover, etc.

A list of suggested projects is as follows:

1. Effluent analysis.
2. A study of the technological details of malt preparation.
3. A study of the technological details of the brewing industry.
4. A study of the organisation of a fermenter.
5. Technological analysis of the process of drug development, drug designing and drug targeting.
6. A study of the technological details of vaccine development.
7. Diagnosis of diseases by modern techniques like ELISA, RIA and Antibody targeting.
8. DNA finger-printing.
9. DNA foot-printing.
10. Microbiological contaminants in food and food products.
11. Isolation of microbes from air, water and soil.
12. Methods of identifying microbes (various staining techniques and biochemical reactions).
13. Tissue Culture and its applications.
14. Stem Cell Technology
15. Nanotechnology
16. Bioinformatics
17. Genetic Engineering
18. Cloning
19. Instrumentation in biotechnology
20. Forensic Biotechnology

Practical File – 5 Marks

The Visiting Examiner is required to assess students on the basis of the practical file maintained by them during the academic year.

LIST OF EQUIPMENT FOR BIOTECHNOLOGY PRACTICALS FOR CLASSES XI & XII

1. Table-top Centrifuge
2. Vortex - Mixer
3. Thermostatic water-bath
4. Spectrophotometer (UV visible range)
5. Refrigerator
6. pH meter
7. Air-dry oven
8. Autoclave (Vertical)
9. Desiccators
10. Micro-filtration unit
11. Chromatography columns
12. TLC Plates
13. DNA gel photographs showing plasmid and chromosomal DNA bands
14. Colourimeter
15. Magnetic stirrer with hot plate
16. Laminar flow cabinet (Vertical)
17. Weighing Balance (Electrical)
18. Hot plate
19. Binocular Microscope
20. Haemocytometer
21. Colony counter
22. Antiserum
23. Antibodies
24. Micropipettes
25. Microcentrifuge

LIST OF ABBREVIATIONS TO BE STUDIED

1. NBTB- National Biotechnology Board
2. DBT- Department of Biotechnology
3. DST- Department of Science and Technology
4. CSIR- Council of Scientific and Industrial Research
5. IARI- Indian Agricultural Research Institute
6. ICMR- Indian Council of Medical Research
7. NBRI- National Botanical Research Institute
8. CIMAP- Central Institute of Medicinal and Aromatic Plants
9. CDRI- Central Drug Research Institute
10. CCMB-Centre for Cellular and Molecular Biology
11. HGRI- Human Genome Research Institute
12. NII- National Institute of Immunology
13. NBPGR- National Bureau of Plant Genetic Resources
14. ICGEB-International Centre for Genetic Engineering and Biotechnology
15. NHGRI- National Human Genome Research Institute
16. IBPGR- International Board of Plant Genetic Resources
17. EBI- European Bioinformatics Institute
18. DDBJ- DNA Databank of Japan
19. EMBL- European Molecular Biology Laboratory
20. BLAST- Basic Local Alignment Search Tools
21. TIGR- The Institute of Genomic Research
22. IEF- Isoelectric Focusing
23. RCF- Relative Centrifugal Force
24. HEPA- High Efficiency Particulate Air
25. CFTR- Cystic Fibrosis Transmembrane Conductance Regulator Gene
26. NCBI- National Centre for Biotechnology Information
27. EST- Expressed Sequence Tag
28. ARS- Autonomously Replicating Sequence

29. GM- Genetically Modified
30. ddNTP- Dideoxynucleoside Triphosphate
31. STS- Sequence Tagged Sites
32. PCR- Polymerase Chain Reaction
33. VNTR- Variable Number of Tandem Repeats
34. RAPD - Random Amplification of Polymorphic DNA
35. RFLP- Restriction Fragment Length Polymorphism
36. PAGE- Polyacrylamide Gel Electrophoresis
37. SCP- Single Cell Protein
38. HGP- Human Genome Project
39. HBsAg- Hepatitis B Surface Antigen
40. Km- Michaelis Menton Constant
41. SSB- Single Strand Binding Protein
42. IFN- Interferon
43. DBM- Diazobenzene Oxymethyl
44. AFLP- Amplified Fragment Length Polymorphism
45. SNP- Single Nucleotide Polymorphism
46. CPU- Central Processing Unit
47. GST- Genome Sequence Tag
48. GDB – Genome Database
49. MGD – Mouse Genome Database
50. YAC – Yeast Artificial Chromosome
51. BAC – Bacterial Artificial Chromosome
52. PDB - Protein Data Bank
53. PIR - Protein Identification Resources

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