

TYBSc Paper III

Biotechnology (90 Lectures)

[This paper consists essentially of two parts: one starting with the homegrown technology for the production of food and beverages that depended on fermentation by microorganisms and the other, of altering the very basic features of living organisms through manipulation of genes. A third component is a logical offshoot of the latter, in the era of information technology: the bioinformatics. Since genetic data in the form of huge number of sequences of nucleotides are made available through molecular techniques, it is now possible to derive maximum knowledge from this enormous amount of data with the help of information processing algorithms. The economic, social and ethical implication of such a major technology too is kept in focus.]

Section I

Food & Fermentation Technology

45 Lectures

[Ancient fermented food processes, such as making bread, wine, cheese, curds, *idli* etc., some of which are some 6,000 yr old, and developed long before man had any knowledge of the existence of the micro-organisms involved, also genuinely constitute biotechnology. However, for the sake of convenience, many people exclude these traditional processes from the realm of biotechnology. Aspects of 'modern biotechnology' may have significant effects on 'traditional biotechnology'. Genetic manipulation to improve brewing and baking yeasts or to introduce new characteristics in crops, biological control of plant pests, and new methods of diagnosing and preventing plant, human and animal disease, are all now realisable. This section, therefore, deals with an introduction to **the integrated use of biochemistry, microbiology and chemical engineering** to exploit plant materials and other genetic resources for the production of specific products and services.]

UNIT I

1. History and development of Food & Fermentation Technology (1 lectures)
2. Fermentation technology & Instrumentation (14 lectures)
 - i. Principles of microbial growth, screening (primary & secondary) and strain improvement (mutation & selection using auxotrophy & analogue resistance)
 - ii. Batch vs Continuous fermentation
 - iii. The Bioreactor / Fermenter & accessories (Stirred tank & Airlift)
 - iv. Media design for fermentation (include molasses, corn steep liquor)
 - v. Downstream processing (use ex of Penicillin and an enzyme? for cell disruption)
 - vi. Instrumentation: Principles and technique of Centrifugation, Spectrophotometry & Chromatography

UNIT II

3. Food and Beverage Biotechnology (9 lectures)
 - i. Technological aspects of industrial production of Cheese, Beer, Vinegar, Single Cell Protein, Mushroom, Yoghurt and Wine (certain details to be dealt with in corresponding practicals)
 - ii. Food quality assurance: Regulatory & social aspects of food biotechnology
4. Enzyme Technology (5 lectures)
 - i. Enzyme production ex. Amylase (bacterial & fungal)
 - ii. Immobilized Biocatalyst (method of immobilization, applications – biosensors)

Unit III

5. Application of fermentation technology in medicine (7 lectures)
 - i. Production of antibiotics (Penicillin)
 - ii. Vitamins (Vit B12)
 - iii. Vaccines (polio, HbsAg)
 - iv. Monoclonal antibodies
 - v. Biopharmaceuticals (Insulin / IFN- α)
6. Application of fermentation technology Agriculture (4 lectures)
 - i. Secondary metabolites from plant tissue culture
 - ii. Biopesticides – bacteria (*B. thuringiensis*), Virus (Polyhedrosis virus) and fungal (*Trichoderma*)
7. Plant and Animal Tissue culture (5 lectures)
 - i. Animal – Laboratory setup, Media, Basic techniques (Disaggregation of tissue and primary culture, maintenance of cell lines- see also Practicals)
 - ii. Plant – Media, Basic techniques (callus and suspension culture, organogenesis, & somatic embryogenesis, Protoplast isolation and fusion)

Section II

GENETIC ENGINEERING (45 lectures)

[The development of genetic manipulation techniques in the latter half of the last century was a breakthrough because not only humankind could create organisms with newer combination of properties, but also it opened up a completely new way of studying biology. At the same time, the field has thrown up newer questions of ethics, morals and environmental safety. Basic understanding of genetic engineering is also important for citizens to make informed choices contributing to public policy.]

Unit IV

1. Introduction to the history of Gene Cloning (1 lecture)

2. Basic methodologies for gene cloning (5 lectures)
 - i. Cutting and Joining DNA molecules: Type I, II, III, Restriction Mapping, DNA Ligase, Homopolymer tailing, Adaptors, Linkers, Use of Alkaline Phosphatase
 - ii. Electrophoresis based techniques: Gel electrophoresis, Southern, Northern and Western blotting.

3. Cloning Vectors (Plasmid, Bacteriophage, Cosmid) (7 lectures)
 - i. Basic properties of Natural and artificial plasmids
 - ii. pBR322 : structure, origin and uses
 - iii. Expression of Insulin and Somatostatin genes in E coli using pBR322.
 - iv. pUC vector
 - v. Special vectors: transcription vectors for probe and ds RNAs
 - vi. Bacteriophage lambda as vector
 - V. M13 vector
 - Vi. Cosmid vector

5. CLONING STRATEGIES (3 lectures)
 - i. Shotgun cloning
 - ii. Making genomic and cDNA libraries in *E.coli*
 - iii. Chromosome walking
 - iv. Chromosome jumping

UNIT V

6. Screening and selection of the desired clone (4 lectures)
 - i. Immunochemical method
 - ii. Nucleic acid hybridization method
 - iii. Subtractive cDNA cloning
 - iv. HRT and HART

7. Techniques for analysis of genes and gene products (5 lectures)
 - i. DNA sequencing by Sanger's, Maxam and Gilbert's methods, Concept of automated sequencing
 - ii. Basic PCR, RT-PCR, and differential display
 - iv. Microarray
 - v. 2D electrophoresis

8. Cloning in eukaryotes: (7 lectures)
 - a) Cloning IN *S. cerevisiae*: basic principle
 - i. Development of vectors: Yep
 - ii. Yeast Artificial Chromosome
 - b) Cloning vectors in plant cells Ti Plasmid, CaMV
 - c) Cloning vectors in animal cells SV 40, Baculovirus

UNIT VI

9. Applications of recombinant DNA technology: (7 lectures)

- a) Transgenic animals and plants (4)
 - i. Xenopus oocyte as an expression system
 - ii. Giant mouse (MMT promoter-growth hormone fusion gene)
 - iii. Drosophila (using p element-mediated techniques-enhancer trap)
 - 9. *Bt* cotton (pesticide resistance gene, weedicide resistance gene)
 - iv. *Knock-out, knock-in and knock-down systems*
 - b) Other important applications: (3)
 - ii. DNA finger printing
 - iii. DNA markers – SNP, VNTR, RFLP, AFLP
- 10. Issues on recombinant DNA technology: (2 lectures)
 - i. Applications in industry- medical/pharmaceutical, agricultural
 - ii Applications in basic research –intellectual property rights and the open source biotechnology movement
- 11. Bioinformatics: (4 lectures- more details will be dealt with in practical)
 - i. Biological Databases
 - ii. Sequence annotation and comparison
 - iii. Multiple sequence alignment
 - iv. Phylogenetic trees

PACTICALS – III BIOTECHNOLOGY

[This practical syllabus should enable the student to understand basic concepts in enzymology, certain methodologies of bioassays and basic techniques in molecular biology and bioinformatics. Costing too is an important component in technology education. The **Good Lab Practices (GLP)** introduced from the first year of the B.Sc. Lifesciences program is expected to be followed with added urgency in this program.]

As the “Expected Learning Outcome” is a good measure of defining the extent of details of an experiment necessary at a certain level, this is incorporated in each case:

- I= Instrumentation knowledge
 - C= Concept understanding
 - T=Technical skill
 - R= relevance
1. Extraction and purification of enzyme: amylase from sweet-potato/salivary amylase/ egg white lysozyme or any other convenient enzyme
 - i) to determine enzyme activity I,C,T, R
 - ii) specific activity. I,C,T
 2. Determination of the Km of amylase/any other convenient enzyme. I,C,T
 3. Determination of the effect of pH/temperature on amylase/any other convenient enzyme activity. I,C,T

4. Immobilization of Amylase/any other convenient enzyme using hen egg-white / alginate method and assay its activity. I,C,T
5. Bioassay of antibiotic/plant extract for anti-bacterial activity. I,C,T,R
6. i. Agarose gel electrophoresis of amylase using serum/ egg white as a control:coumassie blue staining I,C,T,R
ii. Activity staining/Zymogram using starch agar plates. I,C,T
7. Thin layer chromatography of lipids/plant alkaloids/any other suitable extract. I,C,T,R
8. Non-denaturing Poly Acrylamide Gel Electrophoresis of E.coli extract/ Serum proteins/ Saliva/Egg white any other suitable sample I,C,T
9. Extraction of plasmid DNA & Agarose Gel Electrophoresis of plasmid DNA/Restriction Digest with costing of the experiment I,C,T
10. Introduction to databases and bioinformatics: use of public domain/open source database and programs for studying genomics of human/ mouse, yeast/ plant/ microbes or any other relevant organisms I,C,T
11. Manual annotation of DNA sequence: i) pUC series or any convenient cloning/expression vector followed by using programmed tools C,T,R
12. Blast search of genome sequence , Sequence alignment – pair wise / multiple, construction of Cladogram / phylogram I,C,T
13. Assay of fermentation product – Estimation of (a) alcohol/Acetic acid/lactic acid (b) Sugar C,T,R
14. **Any two** of the following open-ended projects:
 - i. Home-Wine production/Home-Vinegar production from any convenient source & assay for fermentation products R,C,T,I
 - ii. Culturing & biomass estimation of mushroom/ *Spirulina* /*chlorella* by cell count/dry weight and estimation of percentage total protein. R,C,T
 - iii. Design & Fabrication of Electrophoresis Chamber R, I,C,T
 - iv. Plant tissue culture: a) Callus production b) Preparation of protoplasts and estimate viability by trypan blue staining T,C
 - v. Growth curve of E coli (DH5 alpha) and preparation of competent cell for transformation experiment. C,R,T,I
 - vi. SDS PAGE with suitable Protein sample for Comparison with Experiment no.9 above. C,T,I

- vii.** Genomic DNA extraction, purification and estimation by UV spectroscopy . I,C,T
- viii.** Costing of DNA extraction protocol & Development of cost effective Method using Liquid Soap, Common Salt and Alcohol or any convenient variation. R,C,T,I
- ix.** Costing of Agarose Gel Electrophoresis of DNA with & without Molecular marker ladder R,C,T,I
- x.** Finger Printing technique using electrophoresis of protein/DNA digest C,T,I,R
- xi.** Animal tissue culture: Tissue dissociation by trypsinization technique and to estimate the viability of cells in physiological saline/MEM at 0 hrs and 2 hrs C,T,R

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