MB 201: Molecular Microbial Physiology and Enzymology

Objective: The main aspect of this paper includes physiology of the bacteria in molecular context. It also includes Enzyme structure and kinetic properties of an enzyme.

	No. of Lectures.
UNIT 1: Molecular tools for studying microbial physiology	06
UNIT 2: Molecular adaptation physiology	14
UNIT 3: Structure of enzyme	12
UNIT 4: Enzyme kinetics	12

Unit-1 MOLECULAR TOOLS FOR STUDYING MICROBIAL PHYSIOLOGY

Ref: A. G. Moat

1.1 Mutant hunts
1.2 Reporter gene
1.3 PCR
1.4 DNA Mobility Shift [Gel Shift and Super Shift]
1.5 Primer extension
1.6 Southern blots
1.7 Northern blots
1.8 Western blots
1.9 Southwestern blots
1.10 Two hybrid analysis

Unit-2 MOLECULAR ADAPTATION PHYSIOLOGY

2.1 Introduction to two component signaling system: **Ref. U. N. Streips**
- 2.1.1 Prototypical two component signaling
- 2.1.2 Spectrum of functions

2.2 Physiology, biochemistry and genetic aspects of oxidative stress response and regulation. **Ref: A. G. Moat**

2.3 Heat shock response **Ref: D. White**

2.4 Nutritional stress and Starvation stress response **Ref: A. G. Moat**

2.5 Biochemistry and Physiology of Adaptation in… **Ref: M. Schaechter**
- 2.5.1 Hyperthermophiles,
- 2.5.2 Extremeacidophiles,
- 2.5.3 Halophiles,
- 2.5.4 Alkalophioles,
- 2.5.5 Radiation resistant microorganisms

2.6 Sporulation in *Bacillus subtilis* **Ref: D. White**

Unit-3 STRUCTURE OF ENZYME

3.1 Protein Structure **Ref: T. Palmer**
- 3.1.1 Determination of primary structures,
- 3.1.2 Determination of secondary and tertiary structures,
- 3.1.3 Determination of protein structure by X-ray crystallography,
- 3.1.4 Investigation of protein structure in solution,

3.2 Mechanism of enzyme actions **Ref: L. Stevens**
- 3.2.1 Proximity and orientation effect,
- 3.2.2 Acid-Base Catalysis,

3.2.3 Covalent catalysis,
3.2.4 Metalloenzymes **Ref: T. Palmer**
3.2.5 Electrostatic catalysis
3.3 Investigation of active site structure **Ref: T. Palmer**

Unit-4 ENZYME KINETICS **Ref: T. Palmer**

4.1 Kinetics of uncatalyzed chemical reaction
4.2 Kinetics of Enzyme catalyzed reactions
4.3 Methods use for investigating kinetics of enzyme catalyzed reaction:
4.3.1 Initial velocity studies
4.3.2 Rapid enzyme catalysis
4.4 Kinetics of single substrate enzyme catalyzed reaction:
4.4.1 Michaelis-Menten equation, its modification and its importance
4.4.2 *Vmax* and *Km*
4.4.3 Lineweaver-Burk plot, Eadie-Hofstee plot, Hans plot, Dixan plots
4.4 Enzyme inhibition kinetics:
4.4.1 Reversible inhibition:
4.4.1.1 Competitive inhibition
4.4.1.2 Non Competitive inhibition
4.4.1.3 Un Competitive inhibition
4.4.1.4 Allosteric inhibition
4.4.1.5 Substrate inhibition
4.4.1.6 Partial inhibition
4.4.2 Irreversible inhibition

4.5 Kinetics of multi-substrate enzyme catalyzed reaction:
4.5.1 Ping-pong reaction
4.5.2 Random-order reactions
4.5.3 Compulsory order reactions

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MB 202: Advances in recombinant DNA technology

Objectives: To impart knowledge to students about the advances, regulations and social impact of Recombinant DNA Technology.

	No. of Lectures.
UNIT 1: Tools of recombinant DNA technology.	12
UNIT: 2 Applications of recombinant DNA technology.	11
UNIT: 3 Transgenesis	12
UNIT: 4 Regulations governing recombinant DNA technology.	09

Unit-1: TOOLS OF RECOMBINANT DNA TECHNOLOGY.

1.1 Genomic libraries:
- 1.1.1 Making a Gene Library **Ref: Glick**
- 1.1.2 cDNA Library **Ref: Primrose**

1.2 Blotting procedures:
- 1.2.1 Southern Blotting **Ref: Primrose**
- 1.2.2 Northern Blotting **Ref: Primrose and Watson**
- 1.2.3 Western Blotting **Ref: Primrose**

1.3 IncQ-group plasmid RSF1010, pMUTIN **Ref: Primrose**

1.4 Cloning in Eukaryotic Microorganisms:
- 1.4.1 Cloning in Yeast and other fungi **Ref: Primrose**
- 1.4.2 *Saccharomyces cerevisiae* Expression systems **Ref: Glick**
- 1.4.3 *Pichia pastoris* and other Yeast Expression systems **Ref: Glick**

1.5 Transfection of Plants
- 1.5.1 Gene transfer to plants **Ref: Primrose**
- 1.5.2 Manipulation of Gene Expression in Plants **Ref: Glick**

1.6 Transfection of Animal cells **Ref: Glick**

1.7 Polymerase Chain Reaction **Ref: Primrose**
- 1.7.1 Starting material and enzymes for PCR
- 1.7.2 Factors influencing PCR
- 1.7.3 Quantitative PCR: TaqMan system, Molecular beacons and Scorpion probes

1.8 Screening of Clones:
- 1.8.1 Screening by DNA Hybridization **Ref: Glick**
- 1.8.2 Immunological Screening **Ref: Primrose**
- 1.8.3 Functional Cloning **Ref: Primrose**

Unit-2: APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY.

2.1 DNA Fingerprinting & DNA Forensics **Ref: Watson**

2.2 Antisense Oligonucleotides as Therapeutic Agents **Ref: Glick**

2.3 Human Monoclonal Antibodies **Ref: Glick**
2.4 Gene Therapy
2.4.1 Human Gene Therapy **Ref: Glick**
2.4.2 DNA Vaccines **Ref: Primrose**
2.4.3 Gene Augmentation **Ref: Primrose**
2.4.4 Gene Therapy for Cancer Cells **Ref: Primrose**
2.5 Plants as Bioreactors **Ref: Primrose and Glick**
2.6 Regulation of Gene Action by RNA Interference **Ref: Watson**

Unit-3: TRANSGENESIS.

3.1 Methods of Transgenesis in Animals **Ref: Glick**
3.1.1 Retroviral Vector Method
3.1.2 DNA Microinjection Method
3.1.3 Engineered Embryonic Stem Cell Method
3.1.4 Use of High Capacity Vectors
3.1.5 Cre-*loxP* Recombination System
3.2 Developing Test Systems using transgenic animals **Ref: Glick**
3.3 Benevolent uses of transgenic animals, fishes and birds **Ref: Glick and Primrose**
3.4 Case studies: Dolly, Knockout Mice **Ref: Tamarin**
3.5 Golden Rice
3.5.1 Golden Rice and Beyond **Ref: Potrykus**
3.5.2 Engineering the Pro-Vitamin A Biosynthetic Pathway in Rice Endosperm **Ref: Xudong**

Unit-4: REGULATIONS GOVERNING RECOMBINANT DNA TECHNOLOGY.

4.1 The Recombinant DNA Dispute **Ref: Tamarin**
4.2 Cartagena Protocol **Ref: Cartagena Protocol**
4.3 Regulations in India **Ref: Regulatory Reforms**
4.3.1 Authorities responsible in India
4.3.2 Regulatory Procedures
4.3.3 Reforms in Regulations
4.3.4 Regulation guidelines
4.4 Development of a Policy for Somatic Cell Gene Therapy **Ref: Glick**
4.5 Case Study:Legalities and law-suits filed for open-field trials of Ice-minus *P. syringae* **Ref: Glick**
4.6 Case study:StarLink Corn **Ref: Glick**
4.7 Open field trials of other Genetically Modified Organisms **Ref: Glick**

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MB 203: Immunology and Immunotechnology

Objectives: This paper focuses on principles behind current immunological research. It also explains some basic techniques for the same. It also throws light on its use in the field of therapeutics.

	No. of Lectures.
UNIT 1: Receptor Biology	12
UNIT: 2 Transplantation and Tumor Immunology	10
UNIT: 3 Immunotechnology	10
UNIT: 4 Immunotherapy	10

Unit 1: Receptor Biology

1.1 The Major Histocompatibility Complex **Ref. Abbas**

- 1.1.1 Discovery of the MHC and its role in immune responses
- 1.1.2 Structure of MHC molecules
- 1.1.3 Genomic organization of MHC
- 1.1.4 Expression of MHC molecules

1.2 T-cell receptor **Ref. Kuby**

- 1.2.1 T cell receptor complex:TCR-CD3
- 1.2.2 T cell accessory membrane molecules
- 1.2.3 T cell activation

1.3 B-cell receptor **Ref. Abbas**

- 1.3.1 The B cell receptor
- 1.3.2 Signal transduction by BCR
- 1.3.3 Second signals for B cells provided by complement receptors
- 1.3.4 Presentation of protein antigens by B lymphocytes to helper T cells
- 1.3.5 Helper Tcell mediated activation of B lymphocytes

Unit 2: Transplantation and Tumor Immunology

2.1 Responses to alloantigens and transplant rejection **Ref. Janeway**

2.2 Cancer and immune system **Ref. Kuby**

- 2.2.1 Cancer: Origin and Terminology
- 2.2.2 Malignant transformation of cells
- 2.2.3 Oncogenes and cancer induction
- 2.2.4 Tumors of immune system
- 2.2.5 Tumor antigens
- 2.2.6 Tumor evasion of immune system

Unit 3: Immunotechnology **Ref. Janeway**

3.1 Detection, measurement and characterization of antibodies and their use
3.2 Isolation of lymphocytes
3.3 Characterization of lymphocyte specificity, frequency and function
3.4 Detection of immunity *in vivo*
3.5 Manipulation of immune system

Unit 4 : Immunotherapy **Ref. Paul**

4.1 Introduction
4.2 A major goal for immunotherapy
4.3 Cellular therapeutics
4.4 Antibody therapeutics
4.5 Engineered antibodies for therapy
4.6 Engineering antibodies for cancer therapy
4.7 The clinical applications of antibodies

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MB 204: GENOMICS, PROTEOMICS AND OTHER "OMICS"

OBJECTIVES: This paper describe a rapidly growing branches of high throughput, large scale biology & maturing scientific discipline like Genomics, Proteomics, Transcriptomics and Metabolomics. This paper includes genome analysis, proteome analysis, and structural, functional & interactional proteomics. It also includes emerging trend of Metagenomics in the field of genomics.

	No. of Lectures
UNIT 1: Genome and Genomics	11
UNIT 2: From Genomics to Proteomics	11
UNIT 3: Proteomics	11
UNIT 4: Other "omics"	12

UNIT 1: GENOME AND GENOMICS **Ref: Primrose**

1.1 Introduction to Genomics: Structural, Functional and Comparative

1.2 Genome Mapping: RFLPs, SNPs, AFLPs

1.3 Genome sequencing methods

1.4 Genome Annotation **Ref: Xiong**

1.5 Comparative Genomics of Bacteria, Organelles and Eukaryotes

1.6 The Human Genome Project **Ref: See '6'**

1.7 The HapMap Project **Ref: See '7'**

UNIT 2: FROM GENOMICS TO PROTEOMICS **Ref: R. M. Twyman**

2.1 Holistic Biology of Microorganisms: Genomics, Transcriptomics and Proteomics **Ref: Ian Humphery**

2.2 Proteome and Quest for Complete Proteome Coverage **Ref: Ian Humphery**

2.3 Protein Separation Techniques: 2-D Gel electrophoresis

2.4 Protein Identification:

2.4.1 Determining Protein Sequence

2.4.2 Mass Spectroscopy

2.5 Techniques for solving Protein structure

2.5.1 X-ray crystallography

2.5.2 NMR

UNIT 3: PROTEOMICS **Ref: R. M. Twyman**

3.1 Interaction Proteomics: Methods of Protein-Protein Interaction

3.2 Functional Proteomics:

3.2.1 Protein Microarray and its Application,

3.2.2 Types and Manufacture of protein chip

3.3 Application of Proteomics: In the field of Medical, Pharmaceutical and Plant Biotechnology

UNIT 4: OTHER "OMICS"

4.1 **Transcriptomics:** Introduction and Cellular transcriptome analysis using Kinetic PCR Assay **Ref: Innis**

RNA level Gene Expression: DNA Micro array Technology and its Application, Printing Technologies **Ref: Primrose**

4.2 **Metabolomics:** Introduction and different levels of metabolomics, Sample selection and Sample handling in metabolomics. **Ref: Primrose**

4.3 **Metagenomics:** **Ref: The Science of Metagenomics**

4.3.1 Introduction and role of microorganisms**.**

4.3.2 Invisible Communities: Global Impact and Microbial Communities.

4.3.3 Why Genomics is not enough?

4.3.4 Metagenomics offer a way forward and Contribution in various fields.

4.3.5 Designing a Metagenomics Project: Sequence based and Function based analysis.

References:

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M.Sc. MICROBIOLOGY PRACTICALS

SEMESTER 2

1. Find out the cellulase activity by using CMC as substrate.
2. To check the Invertase enzyme activity …
    a. By varying the substrate concentration and Data analysis.
    b. By varying enzyme concentration.
3. Find out the effect of inhibitor and type of inhibition on invertase enzyme.
4. Digesting DNA with Restriction Endonuclease.
5. Ligation of DNA fragments.
6. To perform PCR.
7. Imunoelectrophoretic analysis: Agarose gel electrophoresis of sera.
8. ELISA detection of anti-HIV sera.
9. ELISA detection of HBsAg.
10. To study RFLP.
11. DNA extraction from Soil
12. Finding an ORF.
13. EMBOSS application in Genomics.
14. EMBOSS application in Proteomics.