

**SARDAR PATEL UNIVERSITY
VALLABH VIDYANAGAR**



**Programme: MSC (CBCS)
Syllabus with effective from: 2018-19**

**BIOTECHNOLOGY
Semester: III**

PS03CBIT21: Microbial Biotechnology

Unit 1

- Scope of Microbial biotechnology.
- Microbial production and applications of primary metabolites: Citric acid, Ethanol, L Glutamic acid, L Lysine, Vitamins B₁₂ and Vitamin B₂
- Industrially important microbial enzymes: Types, mode of action and industrial applications of microbial amylases and proteases

Unit 2

- Microbial production of therapeutically important products:
 - Antibiotics: Penicillin, Streptomycin
 - Ergot alkaloids : Production by Saprophytic cultivation
- Biotransformations of steroids: Hydroxylations and dehydrogenation, Sterol biotransformations.
- Probiotics and prebiotics: Fundamental aspects and health benefits

Unit 3

- Production of single cell protein from bacteria, fungi and algae: Characteristics, nutritional value and safety, substrates used, process examples, applications.
- Cultivation of edible and medicinal mushrooms: Nutritional and medicinal properties
- Production and applications of microbial exopolysaccharides: Classification. Biological functions, structure and biosynthesis of Xanthan and Alginate, Factors affecting fermentative production of exopolysaccharides and downstream processing(recovery).
- Production of bioplastics

Unit 4

- Physiological characteristics, functions and production of starter cultures for dairy products and fermented foods.
- Microbiology and technology of fermented dairy products:
 - Cheese making: Cheese varieties, manufacture of cheddar cheese, Sources and properties of rennets.
 - Yoghurt making:
 - Beer brewing:

Reference Books :

- Biotechnology: Rehm and Reid.
- Comprehensive biotechnology: Murray Moo Young.
- Microbial Technology vol I and II by Pepler
- Microbiology and technology of fermented foods: R. W. Hutkins. Blackwell publishing.
- Topic related review papers

PS03CBIT22: Genetic Engineering

Unit-I

Concept and importance of Genetic Engineering; General strategies and Steps involved in gene cloning; Extraction and purification of DNA and RNA from bacteria, virus, plant and animal cells; physical and enzymatic methods for cutting DNA; DNA ligase and other enzymes involved in gene cloning; Construction genomic and cDNA libraries; Introduction of DNA into host cells; screening and selection methods for recombinant clones.

Unit-II

Cloning vectors- Basic properties and cloning strategies for vectors derived from Plasmids, λ -bacteriophages, M-13 phage, Cosmids, Fosmids, Phagemids, Phasmids, YAC, BAC, HAC/MAC and viral vectors for Plant and animal cells.

Salient features of expression vectors for heterologous expression in E. coli, Yeast, Insect and Mammalian system, factors influencing heterologous gene expression.

Unit-III

DNA sequencing and sequence assembly: Maxam-Gilbert's and Sanger's methods, Shot gun sequencing, Next generation sequencing strategies for large genomes. DNA mapping and DNA fingerprinting: Physical and molecular mapping, Hybridization and PCR based methods of fingerprinting. Site directed mutagenesis: Methods and applications.

Polymerase Chain Reaction: Principle and basic types of PCR; Reverse Transcription and Real Time PCRs.

Unit-IV

Applications of Genetic engineering in improvement of plants, animals and microbes; Gene editing and its applications; Metagenomics and Metabolic engineering; Gene therapy; Restriction and regulations for the release of GMOs; Biosafety and levels of Physical and Biological containment; The Indian Guidelines for release and use of GM organisms.

Reference Books

Genome 3rd Edition – Brown

Molecular Biotechnology – Glick

Principles of Genetic Manipulation – Old and Primrose

Applied Molecular Genetics – Roger Miesfeld

Biotechnology – H. K. Das

Recombinant DNA – Watson et. al.

Molecular cloning – Sambrook and Russel

From genes to clones – Ernst Whittaker

PS03CBIT23: Enzymology

Unit I

Introduction to enzymology and historical developments in enzymology

Enzyme Structure and classification.

Practical Enzymology: Enzyme Activity, assay, factors affecting enzyme activity, progress curve, rate enhancement, enzyme activators, coenzyme and cofactors,

Enzyme specificity

Enzyme purification: Objectives and strategy, separation techniques, test of purity, case study

Unit II

Enzyme Kinetics:

Chemical reaction kinetics and catalysis

Single substrate kinetics: Equilibrium and Steady state kinetics, significance of K_m , V_{max} & K_{cat} , enzyme efficiency

Multisubstrate kinetics: General rate equation, compulsory order, random order and ping-pong mechanisms and their primary and secondary plots.

Enzyme inhibition and its kinetics: Reversible and irreversible inhibition, competitive, non-competitive and uncompetitive, mixed, partial, substrate and allosteric inhibition.

Thermal kinetics: Effect of temperature on reaction rate, enzyme stability, Arrhenius equation and activation energy.

Unit III

Mechanism of Enzyme Action:

Enzyme mechanisms: Factors affecting catalytic efficiency, Mechanism of Lysozyme, Chymotrypsin, Carboxypeptidase, Restriction endonuclease, Aspartate transcarbamylase. Allosteric enzymes and sigmoidal kinetics: Protein ligand binding, Co-operativity, MWC & KNF models,

Multienzyme enzyme complexes

Unit IV

Methods to study enzymes and its mechanisms

Enzyme engineering: Chemical modification of enzymes: methods of modification of primary structure, catalytic and allosteric properties, use of group specific reagents.

Enzymes in non conventional media, Enzymes as analytical reagents.

Isoenzymes and its physiological significance, Ribozymes and Abzymes

Reference Books:

- Fundamentals of Enzymology : Nicholes C. Price and Lewis Stevens, Oxford Univ. Press.
- Enzyme Structure and mechanism: Alan Fersht, Reading, USA.
- Understanding Enzymes: Trevor Palmer
- The chemical kinetics of enzyme action: K. J. Laidler and P. S. Bunting, Oxford University Press, London.
- Enzymes: M. Dixon, E. C. Webb, CJR Thorne and K. F. Tipton, Longmans, London.
- Proteins: Thomas Creighton
- Biochemistry: Lubert Stryer.

PS03CBIT24: Laboratory I (Practicals based on PS03CBIT21 and PS03CBIT22)

PS03CBIT25: Laboratory II (Practicals based on PS03CBIT23 and PS03CBIT2X)

PS03EBIT21: Advanced Immunology

Unit I

Experimental systems and methods for diagnostics and therapy: Antibody generation (polyclonal, monoclonal, modification of monoclonal antibodies), Methods to Determine the Affinity (*Equilibrium dialysis, surface Plasmon resonance*), Microscopic visualization of cells and sub cellular structures (*Immunocytochemistry, Immunohistochemistry, Immunoelectron microscopy*), Immunofluorescence-Based Imaging Techniques of Antigen-Antibody Interactions (*Flow cytometry, Magnetic activated cell sorting, cell cycle analysis, assays of cell death*)

Antibody Engineering: Chimeric and hybrid monoclonal antibodies, Construction of monoclonal antibodies from Ig-gene libraries

Vaccines: Active and passive immunization, conjugate or multivalent vaccines, DNA vaccines, vaccines under development – malaria and cancer

Unit II

T cell Development: Early Thymocyte Development, Positive and Negative Selection, Lineage Commitment, Exit from the Thymus and Final Maturation, Other Mechanisms That Maintain Self-Tolerance, Apoptosis

B cell Development: The Site of Hematopoiesis, B-Cell Development in the Bone Marrow, The Development of B-1 and Marginal-Zone B Cells, Comparison of B- and T-Cell Development

T-Cell Activation, Differentiation, and Memory: T-Cell Activation and the Two Signal Hypothesis, T-Cell Differentiation, T-Cell Memory

B-Cell Activation, Differentiation, and Memory generation: T-Dependent B-Cell Responses, T-Independent B Cell Responses, Negative Regulation of B Cells

Unit III

Allergy, Hypersensitivity and Chronic inflammation: Allergy: A Type I Hypersensitivity Reaction, Antibody-Mediated (Type II) Hypersensitivity Reactions, Immune Complex-Mediated (Type III) Hypersensitivity, Delayed-Type (Type IV) Hypersensitivity (DTH), Chronic Inflammation

Transplantation immunology: Immunological principles of graft rejection, Role of T cells in graft rejection, Role of Blood Group and MHC Antigens in Graft Tolerance, Predictable clinical course of graft rejection, General and target specific immunosuppressive therapy, Circumstances favoring

Unit IV

Immunodeficiency disorders: Primary and secondary immunodeficiencies

Cancer and immune system: Terminology and Common types of cancer, Malignant transformation of cells, Tumor antigens, The Immune Response to Cancer, Cancer immunotherapy

Tolerance and autoimmunity: Establishment and maintenance of tolerance (*antigen sequestration, central tolerance, peripheral tolerance*), Autoimmunity (*Organ specific autoimmune disease, systemic autoimmune disease, intrinsic and extrinsic factors that can favor susceptibility to autoimmune disease, proposed mechanisms for induction of autoimmunity, treatment of autoimmune diseases*)

Basic Text and Reference Books:

1. Owen, J. A., Punt, J., & Stranford, S. A. (2013). *Kuby immunology* (7th Edn). New York: WH Freeman.
2. Murphy, K., & Weaver, C. (2016). *Janeway's immunobiology* (9th Edn) Garland Science.
3. Male, D., Brostoff, J., Roth, D., & Roitt, I. (2012). *Immunology* (8th Edn) *With STUDENT CONSULT Online Access*. Elsevier Health Sciences.
4. Abbas, A. K., Lichtman, A. H., & Pillai, S. (2014). *Cellular and molecular immunology* (6th Edn) Elsevier Health Sciences.
5. Relevant review articles / research papers / handouts of latest development in the subject.

PS03EBIT22: Bioinformatics

Unit 1:

- ❖ Introduction to Bioinformatics:
 - Overview, Internet and bioinformatics, Applications.
 - Introduction and Bioinformatics Resources:
 - Knowledge of various databases and bioinformatics tools available at these resources, the major content of the databases, Literature databases:
 - Nucleic acid sequence databases: GenBank, EMBL, DDBJ
 - Protein sequence databases: SWISS-PROT, TrEMBL, PIR, PDB, SCOP, CATH
 - Genome Databases at NCBI, EBI, TIGR, SANGER
 - Other Databases of Patterns/Motifs/System Biology (Gene and protein network database and resources)
- ❖ Sequence analysis:
 - Various file formats for bio-molecular sequences: genbank, fasta, gcg, msf, nbrf-pir etc.
 - Basic concepts of sequence similarity, identity and homology, Definitions of homologues, orthologues, paralogues, xenologues.
 - Scoring matrices: basic concept of a scoring matrix, PAM and BLOSUM series.
 - Sequence-based Database Searches: what are sequence-based database searches, BLAST and FASTA algorithms, various versions of basic BLAST and FASTA.
 - Pairwise and Multiple sequence alignments: basic concepts of sequence alignment, Needleman & Wunchsh, Smith & Waterman algorithms for pairwise alignments, Progressive and hierarchical algorithms for MSA.
 - Use of pairwise alignments and Multiple sequence alignment for analysis of Nucleic acid and protein sequences and interpretation of results.

Unit 2:

- ❖ Gene prediction:
 - Gene structure in Prokaryotes and Eukaryotes, Gene prediction methods: Neural Networks, Pattern Discrimination methods, Signal sites Predictions, Evaluation of Gene Prediction methods.
- ❖ Computational RNA Structure analysis:
 - Secondary and tertiary structure of RNA. Various algorithms of RNA folding and their analysis. Energy minimization in RNA folding. RNA sequence alignment based on secondary structure and its applications in functional genomics and phylogeny.
- ❖ Transcriptomics:
 - Complete transcript cataloguing and gene discovery sequencing based approach, Microarray based technologies and computation based technologies

Unit 3:

- ❖ Genomics:
 - Concepts and tools for genomics and comparative Genomics
 - Ancient conserved regions
 - Horizontal gene transfer
 - Functional classification of genes
 - Gene order (synteny) is conserved on chromosomes of related organisms.
 - Prediction of gene function based on a composite analysis.
 - Functional genomics.
 - Putting together all of the information into a genome database.
- ❖ Phylogenetic analysis:
 - Definition and description of phylogenetic trees and various types of trees, Molecular basis of evolution, Method of construction of Phylogenetic trees: Distance based method (UPGMA, NJ),

Character Based Method (Maximum Parsimony and Maximum Likelihood method).

Unit 4:

- ❖ Proteomics and Protein Computational Biology:
 - Tools for proteomics: Acquisition of protein structure information, databases and applications.
 - Structural classification of proteins, Protein structure analysis structure alignment and comparison,
 - Secondary structure and evaluation: algorithms of Chou Fasman, GOR methods.
 - Tertiary Structure: basic principles and protocols, Methods to study 3D structure. prediction of specialized structures.
 - Active site prediction, Protein folding, Protein modeling and drug design
- ❖ Protein structure comparison and classification:
 - Classes, folds, motif, domain; the concepts in 3D structure comparison, purpose of structure comparison, algorithms such as FSSP, VAST and DALI. Principles of protein folding and methods to study protein folding.

Basic Text & Reference Books:

- Bioinformatics: A Beginners Guide, Clavarie and Notredame
- Bioinformatics: David Mount
- Bioinformatics: Rastogi
- Introduction to Bioinformatics: Arthur M. Lesk
- Bioinformatics: Principles and applications, Ghosh and Mallick
- Bioinformatics: Genes, Proteins and Computer, C A Orengo
- Protein Structure Prediction: Methods and Protocols, Webster, David (Southern Cross Molecular Ltd., Bath, UK)

PS03EBIT23: Omics and Computational Biology

Unit I Genomics and methods in genomics

Introduction to the proteome and the genome, codon bias, gene expression, Genome size-C value paradox, DNA sequencing: Maxam- Gilbert, Sanger, Pyrosequencing, automated DNA sequencing. Other features of nucleic acid sequencing. Analysis and Annotation-ORF

Exon-intron boundaries, DNA Microarray technology: The generation of cDNA expression libraries, their robotic arraying, Complex hybridization on DNA chips.

Transcriptomics: Comparative transcriptomics, Differential gene expression; Genotyping/SNP detection; Detection technology; Computational analysis of microarray data.

Unit II Proteomics and methods in proteomics

Relationship between protein structure and function, Identification and analysis of proteins by 2D analysis; Spot visualization and picking; Tryptic digestion of protein and peptide fingerprinting; Common ionization methods for peptide/protein analysis; Introduction to Mass spectrometers; MALDI-TOF and LCMS analyses

Protein-protein interactions: Solid phase ELISA, pull-down assay (using GST-tagged protein), far western analysis, surface plasmon resonance technique, Yeast two hybrid system, Phage display; Protein interaction maps.

Protein arrays-definition, applications- diagnostics, expression profiling. Uses of automated technologies to generate protein arrays and chips.

Unit III Introduction to computational biology basics and biological databases

Computers in biology, Overview of biological databases, nucleic acid & protein databases, primary, secondary, functional, composite, structural classification database, Sequence formats & storage

Pairwise and multiple sequence alignments: Local alignment, Global alignment, Scoring matrices - PAM, BLOSUM, Gaps and penalties, Dot plots. Dynamic programming approach: Needleman and Wunsch Algorithm, Smith and Waterman Algorithm, Hidden Markov Model: Viterbi Algorithm. Heuristic approach: BLAST, FASTA. Building Profiles, Profile based functional identification.

Unit IV Genome analysis

Polymorphisms in DNA sequence, Introduction to Next Generation Sequencing technologies, Whole Genome Assembly and challenges, Sequencing and analysis of large genomes, Gene prediction, Functional annotation, Comparative genomics, Probabilistic functional gene networks, Human genome project. **Structure visualization:** Retrieving and drawing structures, Macromolecule viewing platforms, Structure validation and correction, Structure optimization, Analysis of ligand-protein interactions; Tools such as PyMol or VMD.

References:

- Discovering Genomics, Proteomics and Bioinformatics, A,M, Campbell, C,S,H, Press, (2003).
- Essential of Genomics and Bioinformatics C,W, Sensen, Wiley (2003).
- Hand book of Comparative Genomics: Principle and Methodology by Cecilia Saccone,
- GrazianoPesole, Wiley-LISS publication (2003).

- Proteomics: From protein sequencing to function by S.R. Pennington and M.J. Dunn, Private Ltd (2001).
- Introduction to Proteomics by Daniel C, Liebler, Humana Press.
- Mount, D. W. (2001). Bioinformatics: Sequence and Genome Analysis. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Bourne, P. E., & Gu, J. (2009). Structural Bioinformatics. Hoboken, NJ: Wiley-Liss.
- Lesk, A. M. (2004). Introduction to Protein Science: Architecture, Function, and Genomics. Oxford: Oxford University Press.
- Campbell, M & Heyer, L. J. (2006), Discovering Genomics, Proteomics and Bioinformatics, Pearson Education.

PS03EBIT24: Plant Biochemistry

UNIT I

Structure and biochemical aspects of specialized plant cell organelles – cell plate, primary and secondary cell walls, plasmodesmata, importance of vacuoles, Vacuole and tonoplast membrane, cell wall, plastids and peroxisomes, Characteristics of meristematic Cells.

Water relations of plants: transpiration, guttation, Water balance and Stress Physiology. Osmoprotectants

UNIT II

Photosynthesis - Light and pigments; Light dependent reactions of

Photosynthesis; Carbon metabolism – The Photosynthetic Carbon Reduction (PCR) cycle; Activation and regulation of the PCR cycle, The C4 syndrome, Crustacean Acid Metabolism (CAM), Regulation of C4 photosynthesis and CAM; Translocation and distribution of photo assimilates, Photorespiration, Factors affecting the rate of photosynthesis.

Synthesis and storage of polysaccharide: Starch, sucrose. Fructans and cellulose synthesis as photoassimilates produced by photosynthesis.

UNIT III

Nitrogen metabolism:

Biological Nitrogen fixation by free living and in symbiotic association, structure and function of enzyme Nitrogenase. Nitrate assimilation: Nitrate and Nitrite reductase. Primary and secondary ammonia assimilation in plants; ammonia assimilation by Glutamine synthetase-glutamine oxoglutarate amino transferase (GS-GOGAT) pathway.

Sulfate assimilation and synthesis of sulfur containing substance in plant

Plant Hormones - Growth regulating substances and their mode of action. Role of auxins, gibberelic acid, abscisic acid, cytokinins and brassinosteroids (synthesis and their role)

UNIT IV

Secondary metabolism - Special features, formation and functions of phenolic acids, tannins, lignins, flavonoid pigments, surface waxes, cutin and suberin – the plant protective waxes, terpenes.

Defence system in plants against biotic stresses- roles of phytoanticipins, NADPH oxidase, defense proteins, NO, phenolic compounds, jasmonic acid, ethylene and phytoalexins. Hypersensitive Reaction and Systemic Acquired Resistance (SAR); Induce Systemic Resistance (ISR); Resistance to virus by gene silencing. Genetic basis of pathogen resistance Pathogenesis Related (PR) Proteins.

Reference Books:

Plant Biochemistry by Hans- Walter Heldt; Elsevier Publication

Plant Physiology by Lincoln Taiz and Eduardo Zeiger; Sinauer Associates Inc Publishers

Introduction to Plant Physiology by William G. Hopkins and Norman P. A. Huner; Wiley