

SRI KRISHNADEVARAYA UNIVERSITY:: ANANTAPUR
M.Sc. BIOTECHNOLOGY (SEMESTER SYSTEM)
(Effective from 2005 -2006)

COURSE PATTERN AND SCHEME OF EXAMINATION

Title and Subject of the paper	Work load/per week	Examination	
		Duration (Hours)	Max. Marks
SEMESTER I			
Theory			
Paper I Cell Biology and Microbiology	4	3	100
Paper II Biomolecules	4	3	100
Paper III Biochemical and Biophysical Techniques	4	3	100
Paper IV Biodiversity and Genetics	4	3	100
Practicals			
Practical I Cell Biology, Microbiology and Biomolecules	6	6	100 *
Practical II Analytical Techniques and Genetics	6	6	100 *
SEMESTER II			
Theory			
Paper V Enzymology and Intermediary Metabolism	4	4	100
Paper VI Immunology and Immunotechnology	4	4	100
Paper VII Molecular Biology	4	4	100
Paper VIII Genetic Engineering	6	6	100 *
Practicals			
Practical III Enzymology and Immunotechnology	6	6	100 *
Practical IV Molecular Biology and Genetic Engineering	6	6	100 *

Title and Subject of the paper	Work load/per week	Examination	
		Duration (Hrs)	Max. Marks
SEMESTER III			
Theory			100
Paper IX Plant Biotechnology	4	3	100
Paper X Animal Biotechnology	4	3	100
Paper XI Microbial Biotechnology	4	3	
Paper XII Biostatistics, Bioinformatics and Biosafety	4	3	100
Practicals			100 *
Practical V Plant and Animal Biotechnology	6	6	
Practical VI Microbial Biotechnology, Biostatistics and Bioinformatics	6	6	100 *
SEMESTER IV			
Project		3 months	150
Viva-voce			50
			2000 Marks

* Inclusive of 10 marks for Record and 10 marks for **Viva-voce**

SRI KRISHNADEVARAYA UNIVERSITY:: ANANTAPUR
M.Sc. BIOTECHNOLOGY (SEMESTER SYSTEM)
(w.e.f 2005-2006)

SEMESTER I

Paper I: CELL BIOLOGY AND MICROBIOLOGY

Unit I

Microscopy - Principles of compound, phase contrast, fluorescence, scanning and transmission electron microscopy. Cytophotometry and flow cytometry. Cell structure and organization in bacteria, higher plant and animal cells. Comparison of prokaryotic and eukaryotic cells. Fixation, smearing and staining of cell and microscopic examination of cells. Cell components and organelles – Cell wall, lysosomes, ribosome, golgi complex, peroxisomes, glyoxysomes, mitochondria, plastids, endoplasmic reticulum, vacuoles, centrioles and cytoskeleton. Chromosomes – Nucleosome, Packing of DNA into chromatin. Euchromatin and heterochromatin. Chromatin folding into chromosome. Special types of chromosomes – polytene, lamp brush and accessory chromosomes. Genomes of mitochondria and plastids.

Unit II

Cell division and Cell cycle - Events of cell and cell division in prokaryotes and eukaryotes. Cell cycle regulation. Mitosis and meiosis, and apoptosis. Structure of biomembranes – Chemical composition of animal and plant cell membranes. Assembly of membrane components. Micelle, liposome and biomembranes. Fluid mosaic model of membranes. Membrane transport - Donnan membrane equilibrium, passive, mediated and active transport. Mechanism of active transport (Primary and Secondary – Na^+ , K^+ , Glucose transport). Membrane channels and pumps. Receptors – Chemical nature. Signal transduction – Concept of second messengers (cAMP, Ca^{2+}) and their role.

Unit III

Historical development of microbiology – spontaneous generation, germ theory of disease. General characteristics of protozoa, fungi, algae, cyanobacteria, rickettsiae, mycoplasma, spirochetes and archaebacteria. Outlines of classification of bacteria as per Bergey's Manual of systematic Bacteriology. Nomenclature rules – International code. Chemotaxonomy of bacteria. Nucleic acid based classification of bacteria. Ultrastructure of bacteria – An account of variant (capsule, flagellum, pilus, plasmids, endospore) and invariant (cell wall, cell membrane, mesosomes, nucleoid, ribosome), components of bacterial cell. Morphology and the use in modern biology of *E.coli*, *Bacillus* sp., *Rhizobium* sp., *Agrobacterium* sp.

Nutritional requirements in bacteria – Photoautotrophs, chemoautotrophs, photoheterotrophs, organotrophs, methylotrophs, mixotrophs, auxotrophs. Methods in Microbiology – Different methods of sterilization. Chemotherapy/Antibiotics – Antimicrobial agents, sulfa drugs; antibiotics; penicillin and cephalosporin. Broad spectrum antibiotics. Antibiotics from prokaryotes. Antifungal antibiotics. Mode of action (Penicillin, tetracycline), Resistance to antibiotics. bacterial and fungal toxins. Preparation of culture media. Isolation of pure culture. Culture collection and maintenance of cultures.

Unit IV

Microbial growth – Growth curves, measurement of growth and growth yields. Synchronous growth, continuous culture by chemostats and turbidostats. Growth as affected by environmental factors like temperature, acidity, alkalinity, water availability and oxygen.

Microbial diseases – Diseases, reservoirs, epidemiological terminologies. Tuberculosis. Sexually-transmitted diseases including AIDS. Diseases transmitted by animals (rabies, plague), insects and ticks (rickettsias, lyme disease, malaria), food and water-borne diseases. Pathogenic fungi. Introduction to virology - Nomenclature and classification of viruses, morphological and biochemical properties, ultra structure of TMV, Caulimovirus, M13 and SV40. Lysogeny and lytic cycle in bacteriophage. Biology of sub-viral particles – prions, viroids, satellite viruses, defective interfering particles.

BOOKS RECOMMENDED

CELL BIOLOGY

1. Genes VI. B. Lewin..
2. The Making of a Fly. Lawrence. 1992. Backwell & Scientific Co. Ltd., Oxford.
3. Annual Review of Cell Biology, Krumlaui. 1992. Volume 8.
4. Cell and Molecular Biology, De Roberties & De Robertis
5. The Cell, Vols. I-V, Brachl & Mirsky.
6. Cell and Molecular Biology, Dupre.
7. The Cell, Tawcott.
8. Molecular Biology of Cell, Alberts *et al*
9. Cell Biology, David E. Sadava

MICROBIOLOGY

1. Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. 1986. Microbiology, V edition, McGraw Hill Book Co.
2. Stanier, R.Y., Adelberg, F.A. and Ingraham, J.L. 1984. General Microbiology, IV Edition, McMillan Press.
3. Prescott, L.M., Harley, J.P. and Klein, D.A. 1990. Microbiology, WCB Publishers.
4. Rosenbeng, E. and Chohan, I.R. 1983. Microbial Biology, HS International Editions.
5. Powar, C.B. and Daginwala, H.F. 1989. General Microbiology, Vol. II and I. Himalaya Publishing House.
6. Volk, W.A. and Wheeler, M.F. 1980. Basic Microbiology, J.B. Lippincott Company.
7. Buffaloe, H.D. and Ferguson, D.V. 1981. Microbiology. Houghton Mifflin Company.
8. Freifelder, D. 1987. Microbial Genetics. Jones and Bartlett Publ.
9. Hayes, W. 1988. The Genetics of bacteria and their viruses. John Wiley and Sons.
10. Jacob, F. and Wollman, C.D. 1961. Sexuality and Genetics of bacteria. Academic Press.

Paper II: BIOMOLECULES

Unit I

Chemical foundation of biology – pH, pK, acids, bases, chemical bonding, properties of water. Carbohydrates – Classification and properties of carbohydrates, mono (glucose, galactose, fructose) di (lactose, maltose, sucrose), poly (starch, glycogen, cellulose). Chemical and enzymatic methods for structural elucidation of starch and mucopolysaccharides. Lipids – Classification. Structure and biological functions of fatty acids, triacylglycerols, phospholipids, steroids. Physico-chemical properties and analysis of fats and oils. Structure and functions of prostaglandins, leukotrienes, thromboxanes.

Unit II

Amino acids – Classification. Structure and physicochemical properties. Peptides - Peptide bonds. Naturally-occurring peptides (glutathione, bradykinin, kallikrein, tyrocidin). Peptide synthesis by solid-phase technique. Proteins – Classification. Isolation and purification of protein, criteria of homogeneity. Primary structure of proteins and its sequence determination.

Unit III

Secondary (Ramachandran plot), tertiary and quaternary structural features of proteins, Primary structure and protein folding (Alfensen's experiment on ribonuclease). Forces responsible for protein stability. Structural organization of globular (myoglobin, hemoglobin), fibrous proteins (collagen, keratins, silk fibroin). Denaturation and renaturation of proteins, chaotropic agents. Structure and functions of glycoproteins and lipoproteins.

Unit IV

Nucleic Acids – Structure of purines, pyrimidine, nucleosides, and nucleotides. Structure, properties and functions of nucleic acids (DNA, RNA). Different forms of DNA and RNA. Three dimensional structure of RNA. Isolation of nucleic acids, Denaturation and renaturation of nucleic acids, cot curves, chemical synthesis of DNA. The law of DNA constancy and C-value paradox.

BOOKS RECOMMENDED

1. Textbook of Biochemistry. 1968 by West and Todd (MacMillan).
0. Principles of Biochemistry. 1993 by A.L. Lehninger, Nelson and Cox. (C.B.S., India).
0. Principles of Biochemistry General Aspects. 1983 by Smith *et al.*, (McGraw Hill).
0. Biochemistry (2nd edition) by Donald Voet and Judith Voet.
0. Biochemistry (4th edition) by L. Stryer (Free man).
0. Textbook of Biochemistry with Clinical Correlation (4th edition) by Thomas M. Devlin.
0. Biochemistry by Zubay.
0. Nucleic acid Biochemistry and Molecular Biology by Main Waring *et al.*, (Blackwell).
0. Biochemistry, 2nd edition by Albert L. Lehninger. 1978. Kalyani Publishers, New Delhi.
0. Biochemical calculations, Irwin H.Segel, John Wiley and sons Inc
0. Organic Chemistry, DJ Cram and GS Hammond

Paper III: BIOCHEMICAL AND BIOPHYSICAL TECHNIQUES

Unit I

pH Determination – Measurement of pH, biochemical buffers, selection of biochemical buffer, oxygen electrode and biosensors. Homogenization – Cell disruption methods: mechanical and non-mechanical methods including French press, sonication, freeze-thaw techniques, use of liquid nitrogen in cell disruption. Centrifugation – Basic principles of sedimentation, sedimentation coefficient and Svedberg unit, types of centrifuges and rotors. Preparative ultracentrifugation - differential centrifugation, density gradient, analytical ultracentrifugation and applications in determination of molecular weight, purity and detection of conformational changes in macromolecules.

Unit II

Separation methods - General principles and definitions. Methods based on polarity – Partition chromatography, adsorption chromatography, gas liquid chromatography, reverse phase liquid chromatography. Methods based on size - Gel filtration, principle, methodology and applications. Dialysis, ultra filtration, lyophilization. Methods based on affinity – Affinity chromatography, principle, methodology, applications (purification of proteins, nucleic acids). High performance liquid chromatography – Principle, instrumentation, practical procedure, analysis of results and applications: FPLC, hydrophobic interaction chromatography.

Unit III

Ion-exchange chromatography – Principle, ion exchangers, methodology, pH and salt gradients for elution of proteins, amino acids and nucleotides. Amino acid analyzer. Electrophoresis – General principles and definitions.

PAGE – Native-PAGE, SDS-PAGE, Isoelectric focussing, 2D electrophoresis, identification of novel proteins in 2D gels, capillary electrophoresis.

Agarose gel electrophoresis - Preparation, separation and determination of molecular size of DNA, denaturing agarose gel electrophoresis and their applications, recovery of DNA from agarose gels. Pulse-field gel electrophoresis – principle, methodology and applications in separation of large DNA fragment. Methods to study macromolecular interactions – Mobility shift gel electrophoresis, DNA foot printing, use of fusion proteins to study the protein interactions – GFP, FRET, GST and coimmunoprecipitation.

Unit IV

Spectroscopy – Principles, laws of light absorption, Instrumentation and applications of UV-visible spectrophotometer, fluorescence spectroscopy, NMR, ESR, IR and Raman spectroscopy, Mass spectroscopy – MALDI, TOF. Radioisotope Techniques – Types of isotopes, radioactive decay. Detection and measurement of radioactivity – GM counter, scintillation counter, autoradiography and Cerenkov counting. Preparation of label compounds – pulse chase studies and tracer techniques, isotopes used in biology, safety methods in handling radioisotopes. Optical activity of biomolecules - CD and ORD – principles and applications. X-Ray diffraction – Basic principles, applications in the determination molecular structure of protein and nucleic acids.

BOOKS RECOMMENDED

1. Separation methods in biochemistry by S.J. Morris and P. Morris (Pitman)
2. The tools of Biochemistry by Terrance G. Cooper (Wiley)
3. Biochemical research technique (A practical introduction by Ed. John M. Wriggles worth
4. Analytical biochemistry by David J. Holmes and Hazel peck
5. A Biologist's guide to principles and techniques of practical biochemistry, 2nd edition Ed. by BL. Williams and K. Wilson (Edward Arnold)
6. Biophysical chemistry D. Freifelder, W.H. Freeman
7. Experimental technin. Ex ques in biochemistry by Drewer Pesec, AJ. And As worth, R.B.
8. Principles of Physical Biochemistry by K.E. Vanholdem W.C. Johnson, P.S. Ho, (Prentice Hall), 1998.
9. Manometer and biochemical techniques by W.W Umbrit and R.H. Burris (Burgens
10. The determination of Molecular Structure by P.J. Wheatly (Oxford Clarenders press) 1968
11. Molecular Biophysics – R.B. Setlow and E.C. Pollard (Addison Wiley publishing co. USA)1962
12. Biophysics – Edited by W. Hoppe, W. Lehman Hi. Maskal and H. Ziegler (Springer verlag, Berlin) 1983
13. Practical biochemistry: Principles & Techniques – Edited by Wilson & Walker, Cambridge Univ. Press, New York) 1986.
14. Biological Spectroscopy by Iain D. Campbell and Raymond A. Dwek, the Benjamin/Cumming Pub. Co., California, London.
15. Biophysical Chemistry by C.R. Cantor and P.R. Schimmel, W.H. Freeman & Co., NY.
16. Introduction to Biophysical Methods for Protein and Nucleic Acid Research, Ed. J.A. Glasel and M.P. Deutscher, (Academics Press), 1995.

Paper IV: BIODIVERSITY AND GENETICS

Unit I

Biodiversity - an outline, definition, levels of diversity, animals, plants and microbial diversity, terrestrial and aquatic diversity of genes (gene diversity). Species diversity – Measurement, concept of species, richness, abundance, turnover relationships, global distribution of species, centers of species diversity, mega diversity centers, hot spot analysis. Biodiversity versus biotechnology, biodiversity vs. bioproductivity, bioprospects. Conservation of biodiversity – principle and rationale, *ex situ and in situ*. Conservation - global conservation programs, social movements. Sustainable management of biodiversity, national and international policies.

Unit II

Classical genetics - Outlines of Mendelian genetics. Linkage, crossing over and chromosome mapping in eukaryotes. Numerical and structural variants of chromosome – aneuploidy, polyploidy, deletion, duplication, translocation and inversion. Mutations – molecular basis of mutation and mutagenesis, and Ames test. Extra-chromosomal genetics. Genomes of mitochondria, chloroplast, plasmids and transposable elements (transposons, Ty, Copia, P elements). Molecular mechanism of replicative transposition.

Unit III

Identification of nucleic acids as genetic material. One gene – one polypeptide concept. Modern concept of a gene. Benzer's experiments on r^{II} locus of T₄ phage. Cis-trans test. Concept of cistrons, recones and mutons. Gene clusters, single copy genes and unique sequences. Repetitive sequences in genic and non-genic DNA. Split genes, overlapping genes and oncogenes. Plasmids – types, incompatibility, amplification, curing.

Unit IV

Methods of gene transfer in bacteria: Transformation – Development of competence in *Haemophilus*. *In vitro* transformation – Cold CaCl₂ technique, electroporation. Conjugation - Hfr strains, transfer of F, Hfr chromosomes. Transduction - generalized and specialized. Abortive transduction, cotransduction and sexduction. Use of recombination frequency, interrupted mating and transduction for genetic mapping.

BOOKS RECOMMENDED

BIODIVERSITY

10. K.V.Krishnamurthy. 2003. An advanced text book on Biodiversity, Principles and Practice. Oxford & IBH Publishers. New Delhi.
10. Wilson, E.O and F M Peters (Eds) Biodiversity. National Academy Press Washington.
3. Pushpangadan, P., K.Ravi and V. Santhosh (eds) Conservation and Economic evaluation of Biodiversity. Oxford & IBH Publishers. New Delhi.

GENETICS

1. Stickburgher - Genetics
2. Murray (Ed.) 1991. Gene transfer and expression protocols. Methods in Molecular Biology. Humana Press, Chifton, N.J., pp 439.
3. Lewin, B., Genes VI, Oxford University Press, pp. 857.
4. Suzuki – Principles of Genetic Analysis.
5. Freifelder, D. 1994. Microbial Genetics, Springer.
6. Latchman, D. 1994. Gene regulation (3rd edn.), Sciential Publ.

Practical I: CELL BIOLOGY, MICROBIOLOGY AND BIOMOLECULES

CELL BIOLOGY

0. Microscopic examination of different cell types in plants and animals.
0. Study of mitochondria by vital staining
0. Study of haemolysis using RBC for cell permeability
0. Analysis of mitotic stages
0. Isolation of chloroplast and demonstration of Hill reaction
0. Preparation of haemin crystals
0. Preparation of blood smear and cell count
0. Study of Cheek epithelium
0. Assay of Na⁺- K⁺ - ATPase.
0. Donnan membrane equilibrium.
11. Subcellular fractionation and marker enzymes from rat liver

MICROBIOLOGY

0. Preparation of liquid and solid media for growth of microorganisms.
0. Isolation and maintenance of microorganisms by streaking and serial dilution plating methods.
4. Isolation of pure cultures of microorganisms from soil and water
4. Slants and stab culture. Storage of microorganisms
5. Growth; Microbial growth curve; Measurement of bacterial population by turbidometry and serial dilution methods. Effect of temperature, pH and carbon and nitrogen sources on growth.
6. Microscopic examination of bacteria, yeast and molds and study of organisms by Gram stain, acid fast stain and staining for spores.
7. Assay of antibiotics and demonstration of antibiotic resistance.
8. Analysis for water potability and determination of MPN
9. Bacterial transformation
10. Biochemical characterization of selected microbes

BIOMOLECULES

0. Reactions of amino acids and sugars
0. Isolation and estimation of glycogen from liver
0. Isolation and estimation of Cholesterol from brain
0. Preparation of lecithin
0. Estimation of proteins by Biuret, modified Lowry and Bradford method.
0. Estimation of amino acids by ninhydrin method.
0. Estimation of glucose by glucose oxidase method
0. Titration curve of an amino acid and calculation of pK and pI values.

Practical II: ANALYTICAL TECHNIQUES AND GENETICS

ANALYTICAL METHODS

1. Preparation of buffers and measurement of pH
2. Separation and identification of amino acids by paper chromatography.
3. Separation and identification of sugars by TLC.
4. Separation and identification of lipids by TLC.
5. Separation of amino acids by Ion-exchange chromatography.
6. Separation of proteins by Gel filtration
7. Absorption spectra of amino acids, Proteins and nucleic acids
8. Verification of Beer's law.
9. Determination of molar extinction coefficient of p-nitrophenol.
10. Measurement of inversion of sucrose by polarimetry.
11. Dialysis.
12. HPLC demonstration

GENETICS

1. Bacterial transformation
2. Bacterial conjugation
3. Isolation of auxotrophic mutants
4. Isolation of streptomycin-resistant mutants.
5. Genetics problems based on theory syllabus.

SRI KRISHNADEVARAYA UNIVERSITY: ANANTAPUR
M.Sc. BIOTECHNOLOGY (SEMESTER SYSTEM)
(w.e.f 2005-2006)

SEMESTER II

Paper V: ENZYMOLOGY AND INTERMEDIARY METABOLISM

Unit I

IUB nomenclature and classification of enzymes. Nature of enzymes, Active site, coenzymes, assay of enzyme, units of enzyme activity. Kinetics of enzyme catalysed reaction - Michaelis-Menten equation Determination of V_{max} , K_m , K_{cat} , and their significance. Effect of pH, temperature, concentration of substrate and enzyme concentration, organic solvents on enzyme catalysis and structural consequences. Activators and inhibitors. Enzyme inhibition – reversible and irreversible. Competitive, non-competitive and feed-back enzyme inhibition, enzyme poisoning. Mechanisms of enzyme action – lysozyme, chymotrypsin. Enzyme regulation – Allosteric modification, covalent modification, zymogens activation. Isoenzymes (LDH), Multienzyme, complexes, Multifunctional enzymes, Modern concepts of evolution of catalysis – ribozymes, abzymes

Unit II

Concept of free energy. Thermodynamic principles in biology. Uptake and utilization of nutrients, High energy compounds, Biological energy transducers, Glycolysis, Citric acid cycle, Glyoxylate cycle, HMP shunt, Gluconeogenesis. Components and organization of mitochondrial electron transport system (experimental approach). Mechanism of oxidative phosphorylation. Metabolic pathways involved in production of ethanol, acetic acid, glutamic acid, citric acid and butyric acid.

Unit III

Photosynthetic pigments and photosynthesis in bacteria and higher plants. Chloroplast as an energy transducing organelle. Cyclic and non-cyclic photophosphorylation. Mechanism of photophosphorylation. Pathways of CO_2 fixation by C₃, C₄ and CAM pathways. Photorespiration. Fatty acid oxidation (β -oxidation). Biosynthesis of fatty acids, triglycerides, sterols.

Unit IV

Coordinated synthesis of purine and pyrimidine nucleotides. Synthesis of essential and nonessential amino acids, formation of ammonia and urea. Events triggering secondary metabolite production. Synthesis of vitamins (vit B₁₂ and riboflavin, vitamin A), antibiotics (Penicillin, streptomycin), tannins, alkaloids, saponins and toxins (aflatoxins, endotoxins- BT toxin (ICP)).

BOOKS RECOMMENDED

- 0. Principles of Biochemistry: White. A., Handler, P. and Smith.
- 0. Biochemistry: Lehninger, A.L.
- 0. Biochemistry: David E. Metzler
- 0. Biochemistry: Lubert stryer.
- 0. Review of Physiological Chemistry: Harold A. Harper
- 0. Outlines of Biochemistry: Conn and Stummf.
- 0. Text of Biochemistry: West and Todd.
- 0. Metabolic Pathways: Greenberg
- 0. Mitochondria: Munn.
- 0. Biochemistry: 2nd Edition
- 0. Biochemistry - G. Zubay (1988).
- 0. Mandelstam & Maxuilam – Microbial Metabolism
- 0. Microbiology – Pelczar.

Paper VI: IMMUNOLOGY AND IMMUNOTECHNOLOGY

Unit I

Phylogeny of immune system. Types of immunity - innate and acquired. Cells of the immune system - B cells, T cells, NK cells, phagocytes, inflammatory cells, antigen presenting cells, organs - primary, secondary and tertiary lymphoid organs. Antigens - nature, types, factors influencing antigenicity, haptens, adjuvants and superantigens. Antibodies - structure, types, classes and functions. Antibody diversity – theories of antibody diversity, mechanism of diversification, allelic exclusion.

Unit II

T cell receptor - structure and diversity. MHC - Types, structure, distribution, self restriction, T and B cell activation. Maturation of lymphocytes – positive and negative selection, process of maturation. Antigen processing and presentation – cytosolic and endosomal pathways, T and NK cell - mediated lysis of cells, ADCC. Complement system - components, cascades, MAC, outcomes. Cytokines – classification, properties and role as immunomodulators.

Unit III

Hypersensitivity – classification, mediators, mechanism, consequences of hypersensitive reactions. Autoimmunity - concept of tolerance, types of autoimmune disorders, basis and therapy for autoimmune disorders. Transplantation – transplantation antigens, mechanism of graft rejection, graft versus host reaction, immunosuppressors. Tumor immunity - tumors of immune system, immune responses against tumors. Immunodeficiency - primary and secondary immunodeficiency, combined immunodeficiency, complement deficiency, AIDS.

Unit IV

Antigen–antibody interactions - principle, lattice hypothesis. Precipitation reaction - radial immunodiffusion, Ouchterlony technique, immunoelectrophoresis, counter current and rocket electrophoresis. Agglutination reactions - bacterial and hemeagglutination, passive agglutination, agglutination inhibition assay. RIA and ELISA – principle, methodology and application. Immunofluorescence, FACS, immunoblotting. Hybridoma technology – poly clonals, monoclonals, selection, HAT Medium, production of monoclonal antibodies and applications. Vaccines - concept of immunization, routes of vaccination. Types of vaccines - whole organism (attenuated and inactivated) and component vaccines (synthetic peptides, DNA vaccines, recombinant vaccines, subunit vaccines).

BOOKS RECOMMENDED

1. Essentials of Immunology - Ian & Roitt - Blackwell Scientific Publications.
2. Fundamentals of immunology - William C. Boyed (Wiley Toppan).
3. Introduction to Immunology - John W. Kinball.
4. Fundamentals of Immunology - Otto S. View and others.
5. Immunology - D.M. Wier.
5. Immunology - Jains Kubay, (2001) Second Edition, W H Frecman & Com. New York.
7. Cellular and Molecular Immunology 3rd ed. Abul K. Abbas Andrew K. Lichtman Jordan S. Pober

Paper VII: MOLECULAR BIOLOGY

Unit I

DNA replication – Modes of replication. Messelson–Stahl experiment and Cairns studies. Initiation, elongation and termination of DNA replication in bacteria, eukaryotes and viruses. DNA polymerases, klenow fragment, DNA ligases, topoisomerases. DNA methylation. Regulation of ColE1 plasmid replication. DNA Damage and Repair - DNA damage by UV, alkylating agents, cross linkers. Mechanism of repair – photo reactivation, excision repair, recombination repair, recA in repair, SOS. Adaptive response and its regulation.

Unit II

Transcription (RNA biosynthesis) – RNA polymerases in prokaryotes and eukaryotes. Identification and characterization of promoters. Initiation, elongation and termination of RNA synthesis. Monocistronic and polycistronic mRNAs. Post-transcriptional modifications, RNA replicase in RNA viruses. Polynucleotide phosphorylase reaction, reverse transcription. Inhibitors of RNA synthesis.

Unit III

Translation (protein biosynthesis) – Elucidation of the genetic code, methods employed to decipher genetic code. Activation of amino acids, initiation, elongation and termination of polypeptide chain synthesis. Post-translational modification of proteins. Protein channeling. Inhibitors of protein synthesis.

Unit IV

Regulation of gene expression – Levels of regulation. The operon concept. Enzyme induction and repression. Regulatory genes, structural genes and repressors. Negative and positive regulation. Regulation of *lac*, *ara*, *trp* operons. Regulation of gene expression in lambda and *nif* operon. Regulation of gene expression in eukaryotes – *gal* operon in yeast. Hormones and environmental factors affecting gene expression, antisense RNA and its role in regulation of eukaryotic genes. Molecular biology of cancer, oncogenes, chemical carcinogenesis.

BOOKS RECOMMENDED

- 1 Molecular Biology of the Gene (4th Edn) JD Watson, NH Hopkins, JW Roberts, JA Steitz and AM Weiner, The Benjamin/Cummings Publ, Co. Inc, California.
- 2 Molecular Cell Biology (2nd Edn) J. Darnell, H.Lodish and D. Baltimore, Scientific American Books, Inc. USA 1994
3. Molecular Cloning: A Laboratory manual, J. Sambrook, E.Ffrisch and T. Maniatis, Old Spring Harbor Laboratory Press New York, 2000
4. Introduction to Practical Molecular Biology, P.D. Dabre, John Wiley & Sons Ltd,
5. Molecular Biology, TA Brown (Ed) Bios Scientific Publishers Ltd.,Oxford, 1991

Paper VIII: GENETIC ENGINEERING

Unit I

Genetic engineering - Introduction and outlines of gene cloning. DNA cutting and joining - Enzymatic cleavage of DNA. Restriction and modification enzymes – Classification, nomenclature and importance of restriction endonucleases. Restriction mapping, DNA ligases, polynucleotide kinase, alkaline phosphatases, S1 nuclease, terminal transferase, Bal 31 nuclease. Polymerase chain reaction - principle, types (RT-PCR, nested and inverse PCR) and applications. Sequencing methods – Sanger's, and Maxam-Gilbert's method. Automated sequencing.

Unit II

Cloning vectors - Characteristics of a vector. Natural plasmids used as vectors – advantages and disadvantages. Artificial plasmids and their importance as cloning vectors. Vectors used for cloning in *E.coli*. (plasmids, bacteriophage derivatives, cosmids), BACs, yeast (YAC, shuttle vectors), higher plants (Ti plasmid, caulimovirus) and animal cells (constructs of SV 40 and retroviruses). Characteristics of expression vectors. Generation of DNA fragments containing a gene (shot-gun method, southern analysis, and cDNA synthesis). Joining of DNA fragments to vector molecules, cohesive termini ligation and blunt end ligation - linkers, adaptors and homopolymer tails. Construction of DNA libraries – genomic and cDNA libraries. Screening of recombinants for a positive clone.

Unit III

Introduction of Recombinant DNA molecules into appropriate hosts – Competent cells preparation, electroporation, microinjection and particle bombardment method, and selection of transformants. *Agrobacterium* - mediated transformation of plant cells – Identification of transformed cells and micropropagation of transformed cell into callus, and regeneration of transgenic plants.

Transfection - Salient features and its significance in transforming animal cells. *In vitro* packaging of recombinant cosmids. Introduction of recombinant cosmids into *E.coli* cells. Detection of clones with desired genes - Genetic methods, nucleic acid hybridization methods, colony hybridization, immunochemical methods – ELISA and its significance in identification of clones with desired genes.

Unit IV

Expression of cloned genes - Construction of expression vectors: Vectors having inducible *lac*, *taq* promoters. Use of IPTG and its role in induction of a cloned gene. Expression vector constructed using T₇ promoter. Expression of proteins with His tag and its significance in simultaneous expression and purification of recombinant proteins.

Cloning strategies – Strategies for cloning and over expression of proteins with therapeutic values. Somatostatin, insulin, interferon and growth hormones. Cloning and expression of proteins with immunogenic properties – vaccines. Production technologies – synthesis of plantibodies. Problems associated with expression of cloned genes – inclusion bodies, solubilisation and reconstruction of expressed proteins.

DNA Finger Printing - RAPD, RFLP and AFLP analysis. Markers linked to drug and disease resistant genes. Application of RFLP in forensic science, pedigree analysis, biodiversity, genetic counseling and germ plasm maintenance. Antisense technology and its application. Use of knockout organisms and gene replacement. Use of transposon tagging.

BOOKS RECOMMENDED

1. Molecular Cloning: A Laboratory manual, J. Sambrook, E.Ffrisch and T. Maniatis, Old Spring Harbor Laboratory Press New York, 2000
2. DNA Cloning : a Practical Approach, DM Glover and BD Hames, IRL Press
3. Molecular and Cellular methods in Biology and Medicine. PB Kaufman,W.Wu.D Kim and LJ Cseke, CRC
4. Methods in EnzymologyVol. 152, Guide to Molecular Cloning Techniques, SL Berger and AP Kimmel, Academic Press, Inc San Diego, 1998
5. Methods in Enzymology Vol. 185, Gene Expression Technology, D V Goeddel, Academic Press, Inc. San Diego, 1990
6. DNA Science. A first Course in Recombinant Technology, DA Mickloss and GA Freyer, Cold Spring Harbor Laboratory Press, New York 1990
7. Molecular Biotechnology (2nd Edn) SB Primrose, Blackwell Scietific Pub. Oxford, 1994
8. Milestones in Biotechnology. Classic papers on Genetic Engineering. JA Davies and WS Reznikoff, Butterworth-Heinemann, Boston, 1992
9. Route Maps in Gene Technology, MR Walker and R Rapley, Biackwell Science Ltd, Oxford 1997
10. Molecular Biotechnology – Glick
11. Concepts of Biotechnology D. Balasubramanian
12. Principles of Gene Manipulation by Old and Primrose, Blackwell

Practical III: ENZYMOLOGY AND IMMUNOTECHNOLOGY

ENZYMOLOGY

0. Assay of trypsin
0. Assay of L-amylase from saliva
0. Assay of LDH from serum
0. Assay of acid and alkaline phosphatase
0. A preparation of urease crystals from horsegram seeds and assay
0. Purification of an enzyme and effect of time pH, temperature, substrate concentration, enzyme concentration, inhibition on enzyme activity.
0. Molecular weight determination of enzyme by SDS - PAGE

IMMUNOTECHNOLOGY

1. a. Staining of blood smear and identification of different leukocytes.
b. Determination of A,B, O and Rh blood groups in human beings.
2. Identification of primary and secondary lymphoid organs.
3. Recognition of T-cell by rosette formation.
4. Preparation of antigen.
5. Injection of antigen into experimental animals by different routes.
6. Bleeding of test animals for antiserum.
7. Electrophoretic study of normal and immune serum.
8. Preparation of immunoglobulins from serum.
9. Separation of immunoglobulins by gel electrophoresis.
10. Labelling of purified immunoglobulins with enzymes/dyes.
11. Different serological tests
 - a. Agglutination - Brucella system
 - b. Precipitation test - Ring interface and agar gel double diffusion tests.
 - c. Immunoelectrophoresis - Counter current and Rocket.
 - d. Hemagglutination and Hemagglutination inhibition tests.
 - e. Labeled antibody tests - ELISA, Dot ELISA, FAT and Western blotting.
 - e. Viral infectivity neutralization test.
12. Cell - mediated immunity tests
 - a. Leukocyte migration inhibition test
 - b. Opsonic index.

Practical IV: MOLECULAR BIOLOGY AND GENETIC ENGINEERING

MOLECULAR BIOLOGY

0. Isolation of genomic DNA from bacteria, rice and sheep liver
0. Determination of purity of the isolated DNA by UV spectrophotometry
0. Quantification of DNA
 - . Spectroscopic method (UV absorption method)
 - . Colorimetric method (Diphenylamine reagent)
4. Thermal denaturation of DNA and demonstration of hyperchromic effect.
5. Isolation of RNA by GITC density gradient and hot phenol methods. Estimation of RNA.
6. Thermal denaturation of DNA and demonstration of hyperchromic effect.
2. Determination of melting temperature (T_m) and estimation of GC content.
2. Isolation of plasmids from *E.coli* and separation of CCC, Open circular and linear forms of plasmids
2. Agarose gel electrophoresis – Separation and molecular size determination of DNA
2. Isolation and separation of proteins by SDS- PAGE
2. Southern, Northern and Western blots.
2. Purification of DNA fragment from agarose gels
 - . Electro elution
 - . Affinity purification

GENETIC ENGINEERING

0. Bacterial culture and antibiotic selection media. Preparation of competent cells.
0. Isolation of lambda phage DNA
0. Agrose gel electrophoresis and restriction mapping of DNA
0. Construction of restriction map of plasmid DNA
0. Cloning in plasmid/phagemid vectors – Selection of positive clones using blue/white colours.
0. Preparation of helper phage and its titration using M13 mp18/19 vectors.
0. Preparation of single stranded DNA template
0. DNA sequencing
0. Gene expression in *E.coli* and analysis of gene product
0. PCR
11. Reporter gene assay (Gus/CAT/b-GAL)

SRI KRISHNADEVARAYA UNIVERSITY:: ANANTAPUR
M.Sc. BIOTECHNOLOGY (SEMESTER SYSTEM)
(w.e.f 2005-2006)

SEMESTER III

Paper IX: PLANT BIOTECHNOLOGY

Unit I

Introduction to cell and tissue culture. Tissue culture media (composition and preparation). Initiation and maintenance of callus and suspension culture. Organogenesis, somatic embryogenesis. Shoot-tip culture: rapid clonal propagation and production of virus-free plants. Embryo culture and embryo rescue. Anther, pollen, ovary, ovule, nucellus culture, Endosperm culture for production of haploid plants and homozygous lines. Protoplast isolation, culture and fusion, selection of hybrid cells and regeneration of hybrid plants, symmetric and asymmetric hybrids, cybrids. Cryopreservation.

Unit II

Plant transformation technology - Basis of tumor formation, hairy root, features of Ti and Ri plasmids, mechanisms of T-DNA transfer, role of virulence genes, use of Ti and Ri-plasmids as vectors, binary vectors. Vectorless or direct DNA transfer- particle bombardment, electroporation, microinjection, transformation of monocoats. Mechanism of transgene interaction - Transgene stability and gene silencing. Generation and maintenance of transgenic plants.

Application of plant transformation for productivity and performance – Herbicide resistance - phosphinothricin, glyphosate, atrazine, insect resistance - bt genes, Structure and function of Cry proteins – mechanism of action, critical evaluation of its impact in on insect control. Non-bt like protease inhibitors, alpha amylase inhibitor, virus resistance - coat protein mediated, nucleocapsid gene, disease resistance - chitinase, 1-3 beta glucanase, RIP, antifungal proteins, thionins, RS proteins, abiotic stress – drought and salinity, post-harvest losses, long shelf life of fruits and flowers, use of ACC synthase, polygalacturanase, ACC oxidase, male sterile lines, barstar and barnase systems.

Unit III

Metabolic engineering and industrial products - Plant secondary metabolites. Industrial enzymes, biodegradable plastics, polyhydroxybutyrate, antibodies, edible vaccines. Molecular marker-aided breeding - RFLP maps, linkage analysis, RAPD markers, STS, microsatellites, SCAR (sequence characterized amplified regions), SSCP (single strand conformational polymorphism), AFLP, QTL, map-based cloning, molecular marker assisted selection. Implications of gene patents.

Unit IV

Nitrogen fixation and biofertilizers - Diazotrophic microorganisms, nitrogen fixation genes. Two component regulatory mechanisms. Transfer of *nif* genes to non-diazotrophic microorganisms, *nod* genes structure function and role in nodulation, Hydrogenase - Hydrogen metabolism. Genetic engineering of hydrogenase genes.

Blue-green algae and Azolla - Identification of elite species and mass production for practical application. Mycorrhizae - importance in agriculture and forestry. Algae as a source of food, feed, single cell protein, biofertilizers, industrial uses of algae. Mass cultivation of commercially valuable marine macroalgae for agar agar, alginates and other products of commerce and their uses. Mass cultivation of microalgae as a source of protein and feed.

BOOKS RECOMMENDED

1. J Hammond, P McGarvey and V Yusibov (Eds): Plant Biotechnology. Springer Verlag, 2000
1. TJ Fu, G Singh and WR Curtis (Eds): Plant Cell and Tissue Culture for the Production of Food Ingredients. Kluwer Academic Press 1999
1. HS Chawla: Biotechnology in Crop Improvement. International Book Distributing Company 1998
1. RJ Henry: Practical Application of Plant Molecular Biology. Chapman and Hall 1997
1. PK Gupta: Elements of Biotechnology. Rastogi and Co. Meerut 1996
1. A Text Book of Biotechnology, HD Kumar(WE Pub.)
1. Biodegradation and Detoxification of Environmental Pollutants – Chakrabarthy AM
1. Biotechnology in Agriculture , MS Swamynathan, McMillian India Ltd
1. Gene Transfer to Plants 1995 Polykyus I and Spongernberg, G.Ed. Springer Scam
1. Genetic Engineering with Plant Viruses, 1992 T Michael, A Wilson and JW Davis, CRC Press, PP 384
1. Molecular Approaches to Crop Improvement 1991. Dennis Liwelly Eds. PP 16
1. Plant Biotechnology 1994, Prakash and Perk, Oxford & IBH Publishers Co
1. Plant Cell and Tissue Culture- A Laboratory manual 1994. Reinert J and Yeoman MM Springer

Paper X: ANIMAL BIOTECHNOLOGY

Unit I

History and development of animal tissue culture. Equipment and materials (culture vessels, CO₂ incubator, inverted microscope, cell counters). Principles of sterile techniques. Sources of tissues, types of tissues - epithelial, muscle, connective, nerve and blood. Introduction to balanced salt solutions. Cell culture media - components of the medium, physical, chemical and metabolic functions of media. Role of serum and supplements, serum-free media, features and specifications of MEM, DMEM, RPMI and Ham's medium. Role of antibiotics in media. Measurement of cell number - hemocytometer, coulter counter. Measurement of cell viability and cytotoxicity. Dye exclusion and inclusion tests, colonogenic assay, macromolecular estimation, MTT based assay. Measuring parameters of growth – growth curves, PDT, Plating efficiency and factors influencing growth.

Unit II

Primary culture – Mechanical and enzymatic mode of desegregation, establishment of primary culture. Subculture - passage number, split ratio, seeding efficiency, criteria for subculture. Cell lines - definite and continuous cell lines, characterization, authentication, maintenance and preservation of cell lines. Contamination - bacterial, viral, fungal and mycoplasma contaminations, detection and control, cell transformation – normal vs. transformed cells, growth characteristics of transformed cells. Viral and chemical-mediated methods of cell immortalization

Scale-up of animal cell culture – Factors to be considered. Scale-up of suspension cultures - Batch reactor, continuous culture, perfusion systems. Scale-up of monolayer cultures - roller bottles, Nunc cell factory, microcarrier cultures, organotypic culture, matrices, factors affecting culture and perspectives

Unit III

In vitro fertilization - Concept of superovulation, collection, maintenance, and maturation of oocytes, fertilization of oocytes, Maintenance and assessment of embryos, embryo transfer - Artificial insemination, preparation of foster mother, surgical and non-surgical methods of embryo transfer, donor and recipient aftercare. Cloning - concept of nuclear transfer, nuclear reprogramming and creation of Dolly. Stem cells - embryonic and adult stem cells, plasticity and concept of regenerative medicine. Transgenic animals - retroviral, microinjection, and engineered embryonic stem cell method of transgenesis. Application of transgenic animals - biopharming, disease models, functional knockouts.

Human genome - complexity of the genome, outlines of human genome project, human disease genes. Genetherapy - *ex vivo* and *in vivo* gene therapy methods, applications.

Unit IV

Application of animal cell culture - Vaccine production, specialized cell types. Concepts of tissue engineering - skin, liver, kidney, bladder and heart.

Principles and species suitable for aquaculture (Indian major carps and prawns). Genetic status of culture stocks. Chromosome manipulations - Production of all male and sterile populations, Hypophysation in fishes and prawns. Pearl culture - pearl producing mollusks, rearing of oysters, nucleation for pearl formation and harvesting of pearls. Probiotics and their significance in aquaculture. Molecular tools for the identification of diseases in aquatic species.

Sericulture - species of silkworm, artificial rearing, seed production, biochemistry of silk synthesis, technology of silk production and recent advances.

BOOKS RECOMMENDED

0. Culture of Animal Cells, (3rd Edn) R Ian Fredhney. Wiley-Liss
0. Animal Cell Culture – Practical Approach, Ed. John RW. Masters, Oxford
0. Cell Growth and Division: A Practical Approach Ed. R. Basega, IRL Press
0. Cell Culture Lab Fax. Eds. M Butler & M Dawson, Bios Scientific Publications Ltd. Oxford
0. Animal Cell Culture Techniques Ed Martin Clynes, Springer
0. Methods in Cell Biology, Vol. 57, Animal Cell Culture Methods Ed. Jenni P Mather and David Bames. Academic Press
0. Fish and Fisheries India VG Jhingram
0. Animal Cell Technology, Principles and practices, 1987, Butter, M Oxford press
0. Animal Cell Biotechnology, 1990- Spier, RE and Griffith, JB Academic Press, London

Paper XI: MICROBIAL BIOTECHNOLOGY

Unit I

Fermentation Technology - The component parts of a fermentation process range of fermentation processes, chronological development of fermentation industry. Isolation, Preservation and strain improvement of industrially-important microorganisms. Fermentation media for industrial fermentation.

Design of Fermentor - Basic functions of a fermentor for microbial culture, aseptic operation and containment, Aeration and Agitation, valves and steam traps. Types of fermentation, Types of fermentation vessels.

Fermentation Processes - Batch, fed-batch and continuous fermentations. Solid-state fermentations, Dual or multiple fermentations.

Recovery and purification of fermentation products (down-stream processing) - Recovery of microbial cells, cell disruption. Chromatography, membrane processes, drying and crystallization.

Fermentation Economics - Market potential, plant and equipment, media, aeration and agitation, recovery costs, water usage and recycling, effluent treatment.

Unit II

Enzyme and cell immobilization and their industrial applications.

Industrial Production of

Antibiotics – Penicillin, Streptomycin, Tetracyclines

Organic acids – Citric acid, Lactic acid, Acetic acid.

Enzymes – Amylases, Proteases, lipases

Amino acids – Lysine, Glutamic acid

Beverages – Wine, Beer, alcohol

Vaccines – Rabies, Hepatitis B

Vitamins – Vit B12, Riboflavin

Microbial leaching - Organisms involved in leaching, Chemistry of microbial leaching and commercial process.

Unit III

Introduction to food technology - Elementary idea of canning and packing. Sterilization and pasteurization of food products. Technology of typical food/food products (bread, cheese, idli, yoghurt). Food preservation. Commercial production of SCP.

Environmental pollution - Concept of environment, types of pollution, methods for the measurement of pollution, molecular approaches to environmental management – the problem solving approach, its limitations.

Water Pollution and its control - Water as a scarce natural resource, Need for water management, Measurement of water pollution, sources of water pollution, Wastewater collection. Wastewater treatment – physical, chemical and biological treatment processes.

Unit IV

Microbiology of waste water treatment - Aerobic process - Activated sludge, oxidation ditches, trickling filter, towers, rotating discs, rotating drums, oxidation ponds. Anaerobic treatment. Newer approaches to sewage treatment. Treatment schemes for wastewaters of dairy, distillery, tannery, sugar, antibiotic industries. Microbiology of degradation of xenobiotics in environment - Ecological considerations, decay behavior and degradative plasmids. Hydrocarbons, substituted hydrocarbons, oil pollution, surfactants, pesticides. Bioremediation of contaminated soils and waste land. Biopesticides in integrated pest management.

BOOKS RECOMMENDED

1. Microbial Biotechnology – Glazer and Nikaido 1995
2. Biotechnology – A Text Book of Industrial Microbiology – Crueger and Crueger 2000
3. Principles of Fermentation Technology – Stanbury, Whitaker and Hall 1997
4. Microbial Technology. Vol. I and II, - Pepler and Perlman (Eds).
5. Prescott and Dunn's Industrial Microbiology – Reed (Ed).
6. Concepts in Biotechnology – Balasubramanian, Bryce, Dharmalingam, Green and Jayaraman
7. Microbial Ecology – Fundamentals and Applications – Atlas and Bartha
8. Environmental Microbiology – Mitchall
9. Environmental Microbiology – Grant and Long
10. Microbial aspects of Pollution - Skyes and Skinner

Paper XII: BIOSTATISTICS, BIOINFORMATICS AND BIOSAFETY

Unit I

Basics of measures of central tendencies – Mean, median, mode. Measures of variation. Standard deviation. Skewness and Kurtosis. Hardy–Weinberg equations. Linear regression and correlation – meanings of regression, assumptions, methods of least squares, regression lines, correlation coefficient, computation, properties and interpretation, rank correlation. Probability distributions - Binomial, poisson and normal. Basic concepts and definitions – Students t-test, f-test, Chi ² and X tests. X test for goodness of fit, analysis of variance (ANOVA), assumption, one way classification and two way classification with equal number of operations per cell. Multivariate analysis. Designing of experiments – CRD, RBD, LSD, ANOVA

Unit II

Introduction to bioinformatics - What is bioinformatics, various disciplines of bioinformatics. Genome projects and their use, Human genome project. Applications of bioinformatics. Biological databases - Use of databases in biology: Principles, examples and applications. Primary databases - GenBank, SWISSPROT, PDB. Specialized databases - PFAM, SCOP, PROSITE. Database querying using keywords and search engines. Sequence Analysis I - Concepts of DNA/protein sequence alignments and their importance; sequence alignment methods and programs: BLAST, FASTA. Pairwise sequence alignment versus multiple sequence alignment. Sequence Analysis II - Phylogenetic analysis and tree construction, sequence analysis of nucleic acids: Computational analysis, determining transcriptional analysis, secondary structure prediction; primer designing; genome analysis.

Unit III

Molecular modeling and drug design – Web-based protein structure tools. Structure visualization, classification, alignment and analysis determination of structure of proteins. Predicting protein structure – secondary structure prediction, prediction of 3D structure, Domain structures, screening unknown proteins for predicting important functional domains – DNA binding domains, ATP binding domains, helix, P-turn-helix, leucine zippers, zinc fingers. Genomics, proteomics, metabolomics and pharmacogenomics - Accessing genome information, annotating and analyzing whole genome sequencing – *E.coli*, *Arabidopsis*, Human genome, *C.elegans*. Biochemical pathway databases – Modeling kinetics and physiology.

Unit IV

Biotechnology and Society – Social, ethical and legal aspects of biotechnology. Implications of biotechnology on health, environment, food and sustainable agriculture. Biotechnology in industries – international collaboration, national level policies on biotechnology. Regulatory mechanisms in releasing GMOs. IPRs. Plant breeders rights, WTO, GATT & TRIPS. Bioethics and biosafety regulations.

BOOKS RECOMMENDED

1. Bioinformatics, Andreas D. Baxevanis and B.F. Francis Ouelette, 2/Ed Wiley Inter-Science.
2. Bioinformatics, Des Higgins, Willie Taylor, Oxford University Press.
3. Bioinformatics Computer Skills, Cynthia Gibas and Per Jambeck, Oreilly.
4. Introduction to Biostatistics – Sokal and James Rohlf, 1937
5. Statistical Methods 2-S.P. Gupta (Sultan Chand & Sons, 1979; Fundamentals of mathematical Statistics – S.C. Gupta and K. Kapoor.
6. Statistical methods in Biological and Health Sciences – Jkas. S. Milton and J.O. Tsokan, International students, Edition 1983

Practical -V PLANT AND ANIMAL BIOTECHNOLOGY

PLANT BIOTECHNOLOGY

- 0. Preparation of tissue culture media
- 0. Surface sterilization
- 0. Organ culture
- 0. Callus propagation, organogenesis, transfer of plants to soil.
- 0. Protoplast isolation and culture
- 0. Anther culture, production of haploids.
- 0. Cytological examination of regenerated plants
- 0. *Agrobacterium* culture, selection of transformants, reporter gene (GUS) assays.
- 9. Developing RFLP and RAPD maps

ANIMAL BIOTECHNOLOGY

- 0. Preparation of tissue culture medium and membrane filtration
- 0. Preparation of single cell suspension from spleen and thymus
- 0. Cell counting and cell viability
- 0. Macrophage monolayer from PEC, and measurement of phagocytic activity
- 0. Trypsinization of monolayer and subculturing
- 0. Cryopreservation and thawing
- 0. Measurement of doubling time
- 0. Role of serum in cell culture
- 9. Preparation of metaphase chromosomes from cultured cells.
- 10. Isolation of DNA and demonstration of apoptosis by DNA laddering
- 0. MTT assay for cell viability and growth
- 0. Cell fusion with PEG

Practical VI: MICROBIAL BIOTECHNOLOGY AND BIOINFORMATICS

MICROBIAL BIOTECHNOLOGY

1. Production of alcohol by *S. Cerevisiae*
2. Production of citric acid by *A. niger*.
3. Production of streptomycin by fermentation.
4. Production of wine from grapes.
5. Production of glutamic acid.
6. Production of protease/glucose isomerase by batch fermentation.
7. Preparation of toxoid from a toxin.
8. Immobilization of an enzyme by gel-electrophoresis.
9. Immobilization of whole cells for enzyme/antibiotic production by gel entrapment.
10. Characterization of microbes useful in biodegradable organic matter destruction.

BIOINFORMATICS

10. Familiarization with Windows, UNIX and Internet
10. Database searching (Given name of gene/protein search sequence with key words, download the sequence, locate related literature reference).
3. Use of tools at NCBI, EMBL & SWISSPROT
- 3 BLAST analysis and FASTA analysis
- 3 Pairwise and multiple sequence alignment (CLUSTAWX/W).
- 3 Finding composition of sequence, open reading frames
- 3 Detecting signals, Motifs and repeats in sequence
- 3 Secondary structure prediction of protein sequence
- 3 Phylogenetic tree construction
10. Design of PCR primer

SEMESTER IV: PROJECT WORK