DR. BABASAHEB AMBEDKAR MARATHWADA UNIVERSITY, AURANGABAD.

Syllabus of

M.Sc.I & II Year

Biotechnology

Semester-I to IV

For College level

[Effective from academic year 2015-16 & onwards]

M. Sc. Biotechnology revised syllabus, to be implemented from June 2011

A two year M. Sc. Biotechnology course

1. Syllabus structure is for four semesters, total 1200 marks (96 credits). Each semester consists of four theory and four laboratory courses.
2. Theory examinations would be conducted at the end of odd as well even semesters, practical examination on laboratory courses would be conducted at the end of even semester, between March and May.
3. Practical examination would be of two days for each semester, a pair of examiner would be appointed. Marks secured beyond 80% could be subjected for moderation.
4. Writing business, such as; approach, principle, requirements, in brief procedure for all four laboratory courses must be done on day I, practicals needing incubations should be started on day I itself, however practicals without incubations can be performed on day I or day II, this would be left at examiners discretion.
5. Each examiner would function as external for two courses where as same examiner would function as an internal for other two courses.
6. Each examiner would conduct common viva for both courses, viva may be divided in two days if examiner desires or conducted on day II but no on day I, only.
7. Dissertation is being submitted in lieu of two laboratory courses (Bioinformatics and Tissue Technology), weighing 50 marks (4 credits). Although this is submitted in lieu of stated courses, actual project may or may not be directly related to these two courses. Project should however, be directly related to any of the aspects of sixteen theory courses or remaining fourteen laboratory courses.
8. Dissertation writing should be as a manuscript submitted to "Cell", an international peer reviewed journal. Regional format would not be entertained. Mentor and student, both, are expected to understand the writing style of research paper (full length) published in Journal titled "Cell". The cell word should not be mistaken for cell biology books or any such standard or substandard books.
9. Dissertation would include abstract, introduction, materials and methods, results, discussion, acknowledgments, references in chronological order. The writing should not be less than 4000 words without space, excluding figures and tables.
10. Each centre is expected to purchase permanent mounts, essential instruments and every ingredient required for practicals mentioned in various laboratory courses.
11. Each centre should purchase adequate copies of books mentioned in reference list below theory courses.

Syllabus at a Glance

Paper No.	Title of theory paper	Marks	Credits
Semester I			
I	Biomathematics and Biostatistics	50	4
II	Biomolecules and Bioenergetics	50	4
III	Microbiology	50	4
IV	Inheritance Biology	50	4
LC 1	Based on Paper I	25	2
LC 2	Based on Paper II	25	2
LC 3	Based on Paper III	25	2
LC 4	Based on Paper IV	25	2
Semester II			
V	Molecular Biology	50	4
VI	Enzyme Technology	50	4
VII	Cell Biology	50	4
VIII	Basic Immunology	50	4
LC 5	Based on Paper V	25	2
LC 6	Based on Paper VI	25	2
LC 7	Based on Paper VII	25	2
LC 8	Based on Paper VIII	25	2
Semester III			
IX	Applied Immunology and Virology	50	4
X	Gene Expression and Engineering	50	4
XI	Developmental Biology	50	4
XII	Bioinstrumentation	50	4
LC 9	Based on Paper IX	25	2
LC 10	Based on Paper X	25	2
LC 11	Based on Paper XI	25	2
LC 12	Based on Paper XII	25	2
Semester IV			
XIII	Industrial Technology	50	4
XIV	Recombinant DNA Technology	50	4
XV	Tissue Technology	50	4
XVI	Bioinformatics	50	4
LC 13	Based on Paper XIII	25	2
LC 14	Based on Paper XIV	25	2
LC 15	Dissertation in lieu of two practical course	50	4

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M. Sc. Biotechnology
Paper I – 4 credits
Biostatistics and Biomathematics

Unit I: Elements of mathematics-I

Derivatives: derivative of function, Derivatives of First Principles, Derivatives of inverse, exponential functions and trigonometric functions,

Integration: Methods of Integration: direct integration, integration by parts

Unit II: Elements of mathematics-II

Determinant: determinant of order 2 or 3, expansion of determinant, properties of determinant, Crammer rule

Matrix: Types of matrix, Algebra of matrices, Inverse matrix.

Logarithm : Fundamentals of logarithm, natural logarithm and logarithm to other bases, significance of logarithmic scales

Unit III: Sampling, Data Collection and Presentation:

Introduction to Biostatistics, Common Terms and Notations, Applications.

Sampling: Representative Sample, Sample Size, Sampling Bias and Sampling techniques.

Data Collection and Presentation: Type of Data**,** Method of Collection of Primary and Secondary Data, Methods of Data Presentation, Graphical Representation by Histogram, Polygon, Ogive Curve, Pie Diagram.

Unit IV: Central Tendency:

Measure of Central Tendency : Mean ,mode ,median

Measure of Variability : Standard Deviation, Standard Error Range ,Mean Deviation, Coefficient of Variation, Correlation Coefficient and Regression (Positive & Negative),Calculation of Correlation Coefficient & Regration Coefficient , Linear Regression and Regression Equation, ANOVA One and Two Way Classification.

Unit V: Test of Significance :**& Computer based statistical techniques:**

Test of Significance : F-test , Z-test .T-test and Chi-Square ,Probability Distribution : Binomial , Poison and Normal Distributions.

Computer based statistical techniques:Frequency Table of Single Discrete Variable , Bubble Sort , Computation of Mean , Variance and Standard Deviation, T-test , Correlation Coefficient

References :

1. B.K Mahajan method in Biostatistics Jaypee brother medical pulisher Ltd .india .
2. Richard ah Introduction to Biostatistics prentice hall of biostat

3. Campbell R.C Statistics for biologist, Cambridge University Press,Cambridge
4. Wardlaw,A.C.(1985) Practical Statistics for experimental Biologists
5. Baily N.T.J. Statistical methods in Biology English University press
6. P.S.S. Sunderrao & J.Richard An Introduction to Biostatistics Prentice hall of India pvt.ltd. India
7. Khan, Fundementals of Biostatistics
8. B.K. Mahajan Methods in Biostatistics, Jaypee brothers medical publisher ltd,India
9. Robert sokal and James Rohlf Introduction to Biostatistics W.H. Freeman Press

M. Sc. Biotechnology
Paper II – 4 credits
Biomolecules & Bioenergetics

UNIT I : Fundamentals

Structure of atoms, molecules and chemical bonds (bond strength, cleavage of C-C bond), Stabilizing interactions (Van der Waals, electrostatic, hydrogen bonding, hydrophobic interaction.).

Principles of biophysical chemistry (pH,pKa, titration curve, weak acids, bases) buffer,thermodynamics,(laws, concept of entropy, enthalpy, equilibrium constant, free energy change, free energy change for ATP hydrolysis) , colligative properties.

Bioenergetics; oxidative phosphorylation (ETC) coupled reaction (redox reactions) group transfer, biological energy transducers-Substrate level Phosphorylation.

UNIT II :Carbohydrates

Classification, Composition, Structure, Function and Metabolism of Carbohydrates (Glycolysis, TCA cycle, HMP shunt pathway, Gluconeogenesis, Glycogen Synthesis, Biosynthesis of Starch, Lactose & Sucrose.)[Kinetics of each reaction].

Regulation of Carbohydrate metabolism (with reference to glucose), Metabolic Disorders.(Diabetes,Hypoglycemia,Diabetes as a factor for coronary disfunction,Lactose intolerance).

UNIT III :Proteins

General reactions of amino acids, amino acid Metabolism- Biosynthesis,Degradation, Regulation and Metabolic disorders –Phenylketoneria.

Classification, Composition, Structure {Conformation (Ramachandran Plot examples, Secondary, Tertiary, & Quaternary Structure, Domains,Motifs & Folds.)} and function of Proteins, Stability of Protein Structures, Sequencing of proteins.

UNIT IV : Nucleic Acids

Composition, Structure (including Conformation of nucleic acids (A-, B-, Z-,DNA), t-RNA, rRNA & Ribosomes, Micro-RNA; and Stability of nucleic acid structures) and functions of nucleic acids

Metabolism of nucleotides-.Biosynthesis and Regulation of Purines and Pyrimidines by *de novo* and Salvage pathways,

UNIT V : Lipids, Harmones and Vitamins:

Lipids: Definition, Composition, Classification, Structure, Function, Storage lipids, Membrane lipids, Essential & Non-essential fatty acids, {Good & Bad lipids (Cholesterol)}.

Metabolism of lipids: General reactions, Functions, Biosynthesis and Degradation {fatty acids [oxidation of saturated (α & β) and unsaturated], Triglycerides, Phospholipids, Cholesterol, Prostaglandins}, Metabolic disorders (Triglyceridemia, Nayman Sacchs Disease)

Vitamines:Classification ,Functions,Role in metabolism,Vitamins as co-factors.

Metabolic Disorders –A,B,C,D,K.

Hormones **:** Classification of hormones, Endocrine glands, basic mechanism of hormone action, neuroendocrine regulation [TSH,T_3 ,T_4,] Pitutary gland secreted hormone :Prolactin;Gonadotrophin releasing hormon:LH; role of hormones in reproduction (Estrogen,Testesteron,hCG,FSH,LH), control of fertility(Prolactin,Progesteron,FSH,LH), gametogenesis,human growth hormones , hormonal disorders{Thyroiditis(hypothyroidism, hyperthyroidism),Polycystic Ovarian Syndrome/Polycystic Ovarian Disorder(PCOS/PCOD), Insulin Dependent Diabetes,Phaeochromocytoma.}

References:

1. Cohn & Stump – Outline of Biochemistry Wiley Eastern Ltd.
2. Harpers Review of biochemistry – Prentice Hall
3. Cregnton – Protein Structure & Molecular Properties

A. L. Lehninger, D. L. Nelson & M M Cox – Principles of Biochemistry.

4. Lubert Stryer – Biochemistry
5. David Meltzer – Biochemistry : The Chemical Reactions of living Cells –Academic Press, New York
6. Dixon & Webb –Enzymes
7. J. Jayraman- Practical Biochemistry
8. Plummer. –Practical Biochemistry.
9. Horton; priciples of biochemistry.
10. Hames; Instant Notes in Biochemistry.
11. Holme ; Analytical Biochemist
12. A.C.Deb Fundementals of biochemistry
13. Ramakrishnan , Text book of Medical Biochemistry, Orient Longman
14. Zuby - Biochemistry 4th edition
15. Boyer- Concepts in Biochemistry
16. Cooper -The tools of Biochemistry

M. Sc. Biotechnology
Paper III – 4 credits
Microbiology

Unit I: .Microscopic Techniques:

Differences between prokaryotic and eukaryotic cells

Stain & staining: Classification of stains, staining theories and staining techniques: Negative, Monochrome and Differential Staining (Gram, capsule, spore & acid fast staining).

Unit II: The diversity of the microbial world:

Bacterial taxonomy: conventional, adensonian, and molecular approaches to bacterial taxonomy ,including ribotyping, rRNA sequencing, characteristics of primary domains (from five kingdom system of classification),introduction to diversity among Microorganisms, Survival mechanism and their importance (thermophiles, psycrophils, methanogns, alkalophiles, acidophiles, halophiles,)

Unit III: Microbial growth and control

Definition of growth, ,Bacterial cell division , generation time , specific growth rate mathematical expression of growth Monoauxic, diauxic & synchronized growth curves, various methods to obtain synchronized cultures, Direct & indirect methods of microbial growth assessment,

Effect of environmental factors (solutes, temperature, pH, O_2,) on microbial growth; Control of microorganisms by physical & chemical agents including antimicrobial chemotherapy.

Unit IV: Nutrition and pure culture technique:

Pure culture techniques, principles of microbial nutrition, nutritional classification of microorganisms :autotrophic, heterotrophic ,saprophytic & parasitic microbes, construction of microbial culture media: purpose and type **simple medium** [mineral medium (MS) MS plus carbon, MS plus nitrogen, MS plus carbon plus nitrogen plus supplements] **Complex media** Selective, Enrichment, Differential

Techniques of culture collection: isolation, purification, cultivation & preservation of microbes.

Unit V: Microbial physiology;

Sporulating bacteria, stages of sporulation, cytological and macromolecular changes during sporulation. Spore germination

Microbial toxins: detection and molecular mechanism of action.

Microbial stress response, stress proteins and their role in normal cellular physiology.

Two component system

References:

1. Stenier R.Y et al ., General microbiology Mc Millan Press. Inc.
2. Pelczar ., Reid et al., Microbiology, TMH Publication.
3. Madigan M.T.,et al Brock biology of microorganisms J prenctice hall Inc.
4. Johri B.N Extremeophiles. Springer Verlag, NY
5. Talaro; Foundations in Microbiology.
6. Ananthanarayan; Text book of microbiology. Orient Longman Delhi
7. Cappucinno; Microbilogy – a laboratory manual. 4th ed.
8. Harrigan W.E. , Laboratory methods in Food Microbiology, Academic Press
9. Toratora, Funke & Care Microbiology : An Introduction
10. Salley A.J Fundamental Principles of Bacteriology
11. Atlas R.M. Principles of Microbiology
12. Methods in Microbiology series
13. Bergys Manual Vol 1-4

M. Sc. Biotechnology
Paper IV – 4 credits
Inheritance Biology

UNIT-1: Gene Concept, Mendelist and Extension of Mendelian Principles

A. **Concept of Gene:** Allele, Multiple Alleles, Pseudoallele, Complementation tests.

B. **Mendelian Principles:** Dominance, Segregation, Independent Assortment, Deviation from Mendelian Inheritance.

C. **Extensions of Mendelian Principles:** Codominance, Incomplete Dominance (Partial Dominance), Gene Interactions, Pleiotrophy, Genomic Imprinting, Penetrance and Expressivity, Phenocopy, Linkage and Crossing over, Sex determination, Sex Differentiation, Sex Linkage, Sex limited and Sex influenced characters.

UNIT 2: Mutation and Structural Alterations of Chromosome

A. Mutation: Types, Causes and detection, Mutant types –lethal, Conditional, Biochemical, Loss of Function, Gain of Function, Germinal verses Somatic mutants, Insertional Mutagenesis (Transposon based –biological mutagens).

B. Structural and Numerical alterations of Chromosome: Deletion, Duplication, Inverstion, Transloaction, Ploidy and their genetic implications.

UNIT 3: Microbial Genetics

Methods of genetic transfers –Transformation, Conjugation, Transduction, Sex-duction, Mapping genes by interrupted mating, Fine structure analysis of genes –S Benzes work.

UNIT 4: Gene Mapping Methods

Linkage maps, Tetrad analysis, Mapping with molecular markers, Mapping by using somatic cell hybrids, development of mapping population in plants.

UNIT 5: Extra Chromosomal Inheritance

Inheritance of mitochondrial and chloroplast genes, Maternal Inheritance, Plasmid inheritance.

References:

1. **Principles of Genetics 8th edition, Eldon J. Gardner, Michael J. Simmons, and D. Peter Snustad**, Wiley India Edition (Indian edition).
2. **Molecular Genetics: An introductory Narrative (2nd Edition) Gunther S. Stent and Richard Calendar,** CBS Publishers and Distributors (Indian Edition) –Reprint 2004.
3. **Principles of Genetics, 7th Edition, Robert H Tamarin**, Tata McGraw Hill Edition (Indian Edition) –Reprint 2004
4. **Genetics 5th edition –Strickberger**, Pearsons publisher –Low Price Edition (Indian Edition).
5. Modern Microbial Genetics –Editors Uldis N Streips and Ronald E. Yasbin Wiley –Liss publications, 1991.

M. Sc. Biotechnology
Paper V – 4 credits
Molecular Biology

UNIT I : DNA Repair Mechanisms

Excison ,Mismatch,SOS, Photoreactivation,Recombination repair,Eukaryotic repair Mechanisms.

UNIT II : Recombination

Recombination between heteroduplex,Holiday intermediate,Proteins involved in Recombination,Role of recA,recBCD pathway in E.coli,single strand assimilation in Bacteria.

UNIT III : DNA Replication

Unit of Replication(Replicon : Bacterial,Eukaryotic and Extrachromosomal)

Bacterial Replication is connected to cell cycle,Enzymes involved in replication(DNA Polymerases of E.coli and Eukaryotes) Replication origin and Replication fork,Fedility of Replication.

UNIT IV : Transcription

Prokaryotic transcription: RNA Polymerases, Sigma factor and specificity binding to DNA,Promoters and their consensus sequences , Initiation of transcription,Elongation of transcription, Termination of transcription (Rho dependent ,Rho independent termination, Antitermination) RNA Editing,Splicing

Eukaryotic transcription: RNA Polymerases, types & subunits ,Promoter elements for three polymerases, Activators, Enhancers ,Repressors. Elongation and Termination of transcription.

RNA editing , splicing, polyadenylation.

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UNIT V: Translation

Ribosome, formation of initiation complex, initiation factors and their regulation, elongation and elongation factors, termination, genetic code, aminoacylation of tRNA, tRNAidentity,

aminoacyl tRNA synthetase, translational proof-rea ding, translational inhibitors,post translational modification of proteins

References:

1. Benjamin Lewin -Gene VI,Gene VII,Gene IX,Gene X Oxford University press

2 David Friefieder Essentials of Molecular Biology, jones &Barlett publications

3 J. Kendrew Encyclopedia of Molecular Biology Blackwell Scientific publications.

4 Weaver Molecular Biology

5 J.D.Watson, N.H.Hopkins ,J.W Roberts,et al Molecular Biology of the Gene,

Benjamin Cummings publ.co.inc.,California

6 J.Darnell., et al molecular biology of the cell(2nd edition) Garland Publishing Inc.

7 Meyers R.A (ed) ., Molecular biology and biotechnology.VCH publishers NY Inc.

8 Alberts B et al Molecular biology of the cell. Garland Publishing Inc.

9 Watson J.D ., Recombinant DNA.

10 Malacimski;Essentials of Molecular Biology.

11 Stansfield; Molecular and cell biology.

12 Walker Molecular biology and Biotechnology.

13 Brown T.A Essential of Molecular biology Vol 1 and 2 each.

14 Dale Molecular Genetics of Bacteria

M. Sc. Biotechnology
Paper VI – 4 credits
Enzyme Technology

Unit I Enzymology: an Introduction:

Enzymes as biocatalysts, Theories & Mechanism of enzyme action, specificity of enzyme action (lock and key and induced fit model of enzyme activity), mechanisms of enzyme catalysis, units of enzyme activity, turnover number, activation energy

Types of Enzymes (A. Simple enzymes B. Complex enzymes, multienzyme complex, allosteric enzymes, isozymes; Multi-substrate enzymes; Coenzymes and their role in enzyme action

Classification and nomenclature of enzymes

UNIT II: Experimental Measures of Enzyme Activity

Enzyme induction, active site determination; Initial velocity measurements, detection methods, separation methods in enzyme assays, factors affecting the velocity of enzymatic reactions, reporting enzyme activity data, enzyme stability

Unit III: Enzyme Kinetics and Inhibition

Michaelis- Menton kinetics (Pre-steady state, Steady state, Derivation of M-M equation) Determination and significance of Vmax and Km; Linear plots for enzyme kinetic studies

Enzyme inhibition: A. Competitive inhibition B. Uncompetitive inhibition C. Noncompetitive inhibition and kinetics of these types of inhibitions; Importance of studying enzyme inhibition

Unit IV: Enzyme Immobilization

Introduction; aim of Enzyme Immobilization; effect of immobilization on [a] Physical properties [b] Chemical properties [c] Stability [d] activity of enzyme; Advantages of Immobilization; Limitations of immobilized enzyme; Methods of immobilizations

Carrier matrices, Adsorption of enzymes, Covalent coupling (Functional groups that affects the covalent coupling, use of cyanogen bromide Ethyl chloroformate, Carbodiimide, Glutaraldehyde, 3-aminopropyltriethoxysilane) Entrapment and Encapsulation of Enzymes Crosslinking

Application of immobilized enzymes in the industries,

Unit V Applied Enzymology

Use of enzymes in industries, textile, leather, food, industries. Purification strategies

Use of purified enzymes in biosensors Enzyme sensors for clinical diagnosis, environmental analysis, and other applications of biosensors

Effect of organic solvents on enzyme catalysis, denaturation,.

REFERENCES:

1. Dixon & Webb –Enzymes; Academic press New York
2. A.L. Lehninger- Biochemistry
3. A.L. Lehninger, D. L. Nelson & M M Cox – Principles of Biochemistry.
4. Cohn & Stump – Outline of Biochemistry; Wiley Eastern Ltd.
5. Lubert Stryer – Biochemistry
6. R.L. Foster – The nature of Enzymology; Croom-Helm London
7. Harpers -Review of biochemistry –;Prentice Hall New york
8. R.A. Copeland -Enzymes: A Practical Introduction to Structure, Mechanism and Data Analysis,; John Wiley and Sons Inc.
9. Zuby,Parson, and Vanse –principles of Biochemistry; Wm.C. Brown Publishers
10. J. Jayraman- Practical Biochemistry New age Publishing house, Bangalore
11. Plummer. –Practical biochemistry; TMH New Delhi

M. Sc. Biotechnology
Paper VII – 4 credits
Cell Biology

Unit I

Structural Organization and Function of Intracellular Organelles:

Definition of cell, Diversity of cell size and shape, Structure of prokaryotic and Eukaryotic cells Organization, Structure and Functions of subcellular organelles of(Cellwall,plasmamembrane,cilia,flagella,capsule,pilinucleus, mitochondria, Golgi bodies, lysosomes, endoplasmic reticulum, peroxisomes, plastids, vacuoles, chloroplast,) bacteria, yeast, plant and animal cell.

structure & function of cytoskeleton and its role in motility (actin,myosin,microtubules and intermediate filaments)

Unit II

Membrane structure and function:

Structure of model membrane (plasma membrane,Endoplasmic reticulum,.membrane, nucleus,mitochondrial & chloroplast membrane) lipid bilayer and membrane protein diffusion, osmosis,

Transport across membranes: Types of membrane transport(Active,Passive) Role of carrier proteins, ion channels, ion pumps(Na^+ ,Ca^+ pumps,K^+ pumps and ATPase) Protein sorting Mechanism and regulation of intracellular transport,Cotransport by Symporters and antiporters, membrane potential in membrane transport (electrical properties of membranes).

Unit III

Cell division and cell cycle:

Cell Cycle **(**Mitosis and meiosis) steps in cell cycle, regulation,Molecular control of cell division,

Cellular Mechanisms of Development: Cell differentiation in prokaryotic cells.& Morphogenesis, ,Abnormal cell division – leading to tumor,Cell cell fusion in normal and abnormal cell division,Strategies of Microbes.

Unit IV

Organization of genes and chromosomes:

Defination of genes, Chromosomal organization of genes,Operon, interrupted genes, gene families, unique and repetitive DNA, transposons,

Organization of chromosomes: Definition of chromosomes,structure of chromatin and chromosomes, heterochromatin, euchromatin,Histones Proteins.

Unit V

Cellular Communication and Cell signaling:

general principles of cell communication, , cell adhesion and roles of different adhesion molecules, gap junctions, extracellular matrix, integrins,Neurotransmissions and its regulation, Hormones and their receptors, cell surface receptor, G-protein coupled receptors, signal transduction pathways, second messengers, regulation of signaling pathways, bacterial and plant two-component signaling systems, bacterial chemotaxis and quorum sensing.

References

1 Alberts B et al Molecular biology of the cell. Garland Publishing Inc.

2 Lodish et al., Molecular cell biology.Freeman & company ,New York 1999

3 Gennis R.B Biomembranes- molecular structure and function. Springer.

4 G.Posil ,S.T.Crooke (Eds) mechanism of rceptor regulation. Plenum press,1985

5 DM Prescot; Reproduction in Eukaryotic cells, Academic Press

6 S.F Gilbert; Developmental Biology, Sinauer Associates inc

7 Sheeler; cell and Molecular Biology.

8 Sadava ; cell biology

M. Sc. Biotechnology
Paper VIII – 4 credits
Basic Immunology

UNIT I: Immunity & Antigen

Antigen, Epitopes, Immunogenicity, Antigenicity of a compound, Factors influencing antigenicity, Haptens, Adjuvants, Chemical basis of antigen specificity, Superantigens.

Immunity: Innate, Acquired, Humoral, Cell mediated, Immunization (Active & Passive)

Cells & Organs of Immune System: Primary & Secondary Lymphoid Organs, Lymphatic System, Hematopoisis.

UNIT II: Antibodies & BCR:

Antibody: Basic structure, Fine structure, Classes & their biological activity , Multigene Organization, Recombination, Generation of antibody diversity, Class Switching, Expression of Ig genes, Regulation of transcription , Ig Superfamily , Monoclonal antibody (Chimeric Antibody & Humanized Antibody) & its formation & Applications

B cell Receptor: Structure & Organization.

UNIT III: TCR & MHC:

T Cell Receptor : Structure & Organization,TCR-CD3 Complex, T Cell Rearrangement of TCR & Expression of TCR genes, T Cell accessory molecules & their role in activation of T Cells.

MHC: General Organization & Inheritance, haplotypes, Structure & Organization of Class I & Class II MHC, Polymorphism of MHC, Acceptance & Rejection of Graft, Self MHC Restriction, Alloreactivity of T Cells, Foetus as Unrejeced Graft.

UNIT IV: Lymphocyte Activation & Regulation , Effector Mechanism:

T Cell: Maturation in Thymus, Positive & Negative Selection in Thymus, Activation by interaction with Antigen Presenting Cells, Signal transduction, Differentiation & Maturation of T Cells, Clonal anergy & Mechanism of Tolerance.

B Cell: Maturation, T Dependant & Independent Activation, Germinal Centers, Ag induced B Cell Differentiation, B Cell Tolerance.

Effector Mechanism: Cytokines, their properties, receptors, TH1 & TH2 balance, Regulation of Cytokine Synthesis, Cell Mediated Effector Mechanism, Mechanism of Cytolysis,

Complement: Function, Complement Activation, Regulation of Complement System

UNIT V: Antigen-Antibody Interaction:

Strength of Antigen-Antibody Interaction (Antibody Affinity & Antibody Avidity),

Precipitation: Precipitation in Fluids, Precipitation in Gel (Radial Immunodiffusion & Double Immunodiffusion), Immunoprecipitation.

Agglutination: Hemagglutination, Bacterial Agglutination, Passive Agglutination.

Radioimmunoassay, Enzyme-Linked Immunosorbent Assay, Immunofluorescence, Flow Cytometry & FACS, Mixed Lymphocyte Reaction, Cytotoxicity Reaction, In situ localization by techniques such as FISH & GISH, Migration Inhibition Assay.

References:

1. Roitt I M, Essential Immunology , Blackwell Scientoific Publicatuions,Oxford
2. WeissmanI L Wood, Immunology, Benjamin Cummings
3. Kuby - Immunology ,4th ed Freeman press
4. Stites DP Basic & Clinical Immunology, Appleton & Lang press
5. Ellis, Vaccines, A new approach to Immunology
6. W E Paul, Fundemental Immunology, Raven Press
7. D M Weir Experimental Immunology 4 volumes
8. William Paul Fundementals of Immunology
9. Abbas- Cellular and Molecular Immunology
10. Rose- Manual of Clinical and Laboratory immunology
11. Benjamini- Immunology :A short Course
12. Brooks – Medical Microbiology 21st ed
13. Joshi – Immunology
14. Janeway -Immunobiology

LC 1 -Practicals based on Theory Paper I -2 credits

Practicals:

1. Representation of statistical data by histogram ogive curves and pie diagram.
2. Measure of Central tendencies : Arithmatic Mean , median and mode
3. Calculation of Measure of Dispersion : Mean deviation , Standard deviation and coefficient of variation , Quartile deviation .
4. Test of Significance : Chi-square test , t- test , Standard error

LC 2 -Practicals based on Theory Paper II -2 credits

Practicals

1. Preparation of buffers applying HH equation
2. Estimation of pKa values of amino acids
3. Demonstration of colligative properties
4. Estimation of carbohydrates by qualitative methods
5. Estimation of carbohydrates by quantitative method (DNSA / Anthrone / GOD-POD)
6. Purification of polysaccharides
7. Estimation of proteins –(Folin Lowry / Biurets method,Bradford)
8. Determination of isoelectric pH of proteins / aminoacids
9. Isolation of proteins- casein from milk / hemoglobin (from RBC) / pulses
10. Estimation of DNA
11. Denaturation & renaturation kinetics of DNA
12. Estimation of RNA
13. Acid values Iodine number& Saponification values of fats (commercial samples)
14. Isolation and purification of lipids from microbes and eukaryotes
15. Simple assays for vitamins and hormones
16. Preparation / isolation of biomolecules from natural resource (Starch, glycogen, Lecithin, Cytochrome

LC 3 -Practicals based on Theory Paper III -2 credits

Practicals

1. Staining and Microscopic examination of microorganisms (bacteria, Yeasts & molds): Gram staining, acid fast staining, negative staining & other methods
2. Isolation of pure cultures of microorganisms by different plating techniques & serial dilution methods from soil water and air
3. Storage & preservation of microorganisms
4. Growth curve of microorganisms
5. Micrometry
6. Measurement of bacterial population by turbidometry, serial dilution ,methods
7. Effect of temperature, pH on microbial growth.
8. Biochemical characterization (IMViC) of selected microorganism
9. Assay of antibiotics

LC 4 -Practicals based on Theory Paper IV -2 credits

Practicals:

1. Study of one factor cross
2. Study of two factor cross
3. Study of three factor cross
4. Isolation of plasmid DNA
5. Isolation of Chloroplast / Mitochondria -DNA
6. Fluctuation test
7. Isolation of antibiotic resistance spontaneous mutant
8. UV inducted mutagenesis
9. UV survival curve
10. Mutagenesis with Ethidium bromide/ nitrous acid/ hydroxyl amine/ NTG or EMS.
11. Survival curve with chemical mutagen.

SEMESTER 2nd

LC 5 -Practicals based on Theory Paper V -2 credits

Practicals

1. Spontaneous mutation in bacteria
2. Induced mutation using chemical and physical mutagens
3. Scoring and enrichment of mutants
4. Ampicillin enrichment of Auxotrophs
5. Isolation of different auxotrophic mutans by using selective plates
6. Chromosomal abberation due to radiations
7. Repair mechanisms in E.coli –dark,photoreactivation
8. Repair mechanisms in Yeast
9. Study of genotypes and its conformation

LC 6 -Practicals based on Theory Paper VI -2 credits

PRACTICALS

1. Enzyme production from microbes and seeds
2. Enzyme purification by salting out
3. Effect of enzyme parameters on activity
4. Enzyme kinetic analysis (Determination of V_{max} and K_m ,reciprocal plots)
5. Effect of inhibitors on enzyme activity
6. Immobilization of enzymes and study of different parameters of immobilized enzyme preparation

LC 7 -Practicals based on Theory Paper VII -2 credits

Practicals

1. Transport across membranes.
2. Effect of detergents on membrane permeability.
3. Isolation of cellular organelles.
4. Study of marker enzymes from the isolated organelles.
5. Preparation of liposomes.
6. Preparation of Feulgen-Stained Chromosomes in root tip squashes for the observation of effect of Colchicine on Chromosome movements during Mitosis.

LC 8 -Practicals based on Theory Paper VIII -2 credits

Practical:

1. Study of Immune Cells TLC/DLC
2. Isolation of PBMC FROM Heparinised Blood
3. Enrichment of T & B Cells
4. E-Rosseting for T Cells
5. Reverse Plaque Assay for B Cells
6. Isolation of Bacterial Antigen
7. Isolation of Protein A from *Staphylococcus aurous*
8. Immunoelectrophoresis
9. Antigen-Antibody Interaction: Precipitation (In Liquid & In Gel)
10. Haemagglutination
11. Complement Activity on RBC
12. Bactericidal Assay

End First Year

M.Sc. Biotechnology

Paper IX

Applied Immunology & Virology

UNIT I: Immune Response:

Phagocytosis- process of phagocytosis, phagocytic cells, antimicrobial and cytotoxic activities in phagocytic killing- oxygen dependent and oxygen independent killing mechanism.

Antigen Processing & Antigen Presentation – Antigen presenting cells, MHC restriction, Processing and presentation of Endogenous antigen, Exogenous antigen & Non-peptide Bacterial antigen.

A: Appropriate Immune Response:

Immune response to infectious diseases including diagnostic immunology

Bacterial – Host immune response to bacterial infection and bacterial evasion mechanisms(Tuberculosis)- Tuberculin test , IgG, IgA, IgM antibody

Parasite(Malaria) – pathogenesis of Plasmodium species and host response to plasmodium infection, immunoresponce difficulties (pleomorphic nature and undulant appearance of malarial pathogen in smear , no PCR, antibody kits for malaria)

Viral (HIV) - mechanisms of humoral and cell mediated immune responses to viruses, viral evasion of host defense mechanisms.(ELISA, Western blot method, PCR)

Inflammation- As mechanism of protection, Cell Adhesion Molecules, lymphocyte extravasation, Lymphocyte Homing Mediators of Inflammation, Process of Inflammation (Acute and chronic inflammatory response), Role of Granulocytes in the process of Inflammation & Anaphylaxis . Anti inflammatory agents –(Biological or anti-inflammatory immune components)

B: Inappropriate Immune Response:

I. **Autoimmunity**: Mechanism, Organ specific and systemic autoimmune diseases, Diagnosis & Control, proposed mechanisms for induction of autoimmunity.

II. **Immunodeficiency Disease**: Primary & Secondary(AIDS) Deficiencies, Diagnosis & Treatment

III. **Hypersensitivity:** Types & Significance.

UNIT II: Vaccines:

Immunization (Active & Passive), Designing of Vaccines, Attenuated Vaccines (Viral / Bacterial), Inactivated (Viral / Bacterial), Polysaccharide Vaccines, Toxoid Vaccines,

Recombinant Antigen Vaccines, Recombinant-Vector Vaccines, DNA Vaccines, Synthetic Peptide Vaccines, Multivalent Subunit Vaccines.

Production preservation, structure and impact of polyvalent vaccines

Hybridoma: generation with HAT selection, Application of hybridoma technology chimeric antibodies and generation of Humanized antibody

UNIT III: Cancer:

Genetic Rearrangement in Progenitor Cells, Oncogenes, Tumor Suppressor Genes, Cell Cycle of cancerous cell, Virus Induced Cancer-Provirus theory, Metastasis, Interaction of Cancer Cells with Normal Cells, **Apoptosis - Programmed Cell Death*,** Therapeutic Interventions of Uncontrolled Cell Growth. MAb for cancer treatment, flowcytometry, detection of cancer marker proteins in the serum.

UNIT IV: General Virology & Bacteriophage:

Classification, Nomenclature, General Properties, Morphology, Ultra Structure, Types of Envelopes & Composition of *Viruses*

Cultivation, Purification & Enumeration of Viruses, Viriods & Prions.

Structure & Organization of Bacteriophage.;

Genome Organization (Molecular Level), Infection, Multiplication, Replication of T_4 *Phage,* λ *Phage,* M_{13} *Phage, Mu Phage*;

Genetic Switch of λ Phage.

UNIT V: Animal & Plant Viruses:

Animal Viruses: Genome Organization & Replication of Arthopod, Retro, DNA (Adeno, Pox, SV40, Vaccinia, Hepatitis Viruses), Influenza.

Plant Viruses: Genome Organization & Replication of TMV, Cauliflower Mosaic, Potato, Gemini Viruses.

References:

(exact titles to be communicated, at present the authors are listed)

Kubey
Roitt-Essential immunology
Anantnarayan Textbook of microbiology
Tizzard
Genaway
Genomes 3
S.E. Luria General Virology
R.E.F. Mathews
Davis -Microbiology

Notes for teachers:

*this topic should be mentioned only in this paper. Details of this point will be dealt in detail in other paper (paper XI Developmental biology)
there are about 20 that are considered as marker proteins-emphasize on PA125, CA199, BRAC1 AND BRAC2. Rest should only be enlisted

M. Sc. Biotechnology,
Paper X
Gene Expression and Genetic Engineering

Unit I -Gene Expression in Prokaryotes

Cis element and Trans Factors, Operon concept, Co-ordinated control of structural genes, the *lac, trp, ara, gal* operons, repressor proteins; gene /genetic system specific repressor, global regulator, operator sequences, and other DNA elements for the regulation or gene expression (regulatory sequences –DNA and RNA), attenuation mediated regulation –biosynthesis of amino acids, antitermination mediated regulation –phage lambda N and Q as paradigm, negative regulation, catabolite repressor –an example of positive regulation, catabolite repression on non carbohydrate molecules such as tryptophan sysnthesis and degradation (metabolism), stringent response, role of ppGpp in regulation, stationary phase sigma and nitrogen fixation –*nif* genes of *Klebsiella*, regulation of nitrogen fixation in *Rhizobium.*

Unit II Gene Expression in Eukaryotes

Transcriptional activators as positive regulators, TAFs as an example of both activator and repressors, co-ordinated control of expression by different factors, independent domain of protein binding to DNA to activate transcription, Zinc finger motif, Leucine Zipper, Helix Loop helix, Helix Turn Helix –from lambad to eukaryotes, Homeodoain, Upstream activating sequences as cis acting elements and specific factors binding to such UAS –with an appropriate example, Response Elements, Metallothionine regulatory elements as paradigm of multiple level control of activation of the basal apparatus, Identifying genes under common regulation. Role for DNA modification in control of gene expression, Mechanisms of Chromatin remodeling with reference to activation or repression of a region on chromosome, Methylation and Demethylation, Histone deacetylation, acetylation, Insulators, genetic Imprinting, telomere structure and role in regulation of gene expression and cancer progression. Regulation of gene expression at a step subsequent to transcriptional activation e.g TAR sequence of retrovirus, posttranscriptional regulation GCN4 regulation, Non-stop and Missense translation, tmRNA mediated release of mis-sense translation.

Unit III Isolation, Identification and Characterizaiton of DNA Fragments – Ingredients of Genetic Engineering

Systems safeguarding DNA , mechanism of escaping restriction, modification, restriction, criteria for decision of DNA fragment to be restricted or modified, Classification of Restriction Endonucleases, their properties and specificities, applicability.
Modification Enzymes: Each of the enzyme is to be studied with reference to its source, structure, function (activity –mode of action), specificity, reaction conditions, kinetics, and

applications in detail; DNA Ligase, S1 Nuclease, Bal31 Nuclease, Mung bean nuclease, Exonulease III, Exonuclease V, Exonuclease VII, Terminal DNA Transferase, T4 DNA Polymerase, dNA Polymerase I, DNA polymerase II, DNA Polymerase III, Klenow fragment, Taq DNA polymerase, *pfu* polymerase, T7 DNA polymerase, Sequenase, Polynucleotide Kinase, Phosphatases, Reverse Transcriptase, RNaseA, RNaseT, RNaseH.

Unit IV Vectors to incorporate recombinant DNA from homologous or heterologous source

IMP: All the vector biology must cover: structural organization, general features, regulatory features, convenience with reference to restriction sites and cloning orientation, markers, reporters –wherever available, both –merits and demerits and their derivatives

General aspects: Natural plamids; colE1, RSF1030, cloDF13, R6K, F, R1, EntP 307. Properties of plasmid to be a vector; markers, replicons, convenient restriction endonuclease recognition site for cloning.

Plasmid vectors: Construction of pBR322, negative selection or gene disruption strategies, tet promoter, anti tet promoter, restriction map of pBR322, improvded vectors derivatives of pBR322 such as pUC18, pUC19.

Phage Vectors: Phage lambda, genetic organization favoring its subjugation as vector, regulatory circuits, lambda biology, insertion vectors, replacement vectors, vectors with improved properties, invitro packaging –genetics and significance in vector world (**must include list of vectors both from principles of gene manipulation and genomics by Twyman and Primrose 7th edition and from Genes to Clones –by Winnacker).**

M13 phage; biology of phage m13, M13 mp1, M13 mp2, M13 mp18 and M13 mp19 vectors.

Cosmid Vectors: In vitro packaging, cloning schemes for various purposes, pJB8 and c2XB based cloning strategies. Phagemid Vectors; pBLUEscript, phage lambda ZAP series.

Cloning Vectors with high capacity: P1, BAC, YAC

An overview of vectors with reference of cloning size and their merit –demerit.

Vectors for Specialized purposes: M13 for single strand preparation, Expression vector for production of gene products of homologous as well heterlogous origin, vectors for preparation of RNAa probes, interfering RNA, vectors for maximizing expression from cloned rDNA, Vectors with purification tage (teach not less than five different available tags for recombinant protein purification), vectors promoting solubilizaiton of expressed protein, vectors secreting recombinant product, GATEaway system vectors, Pin point vectors.

Animal Vectors: Markers, reporter genes, promoters, gene construct, position effectoptimization of codon, stable gene expression, transient gene expression. Plasmid vectors as pDV2-dhfr, pRSV-neo.

Viral Vectors: Runaway polyoma replicons, BK, BPV, Epstin Bar virus, replicon based vectors, Adeno virus vectors, adeno associated virus vectors, Baculovirus vectors, Herpes virus vectors, Retrovirus vectors, Lentiviral vectors, Sindbi virus vectors, Vaccinia virus and pox virus based vectors, P-element based vectors.

Plant Molecular Biology Vectors: *Agrobacterium tumafaciens* based plasmid Ti, overview of plasmids producing octopine, nopaline, phenolic signaling molecules, Disarmed Ti plasmid vector, control of transgene expression in plants, selection markers, reporter genes in plant molecular biology, Binary Vectors, pGreeenII, 35s CaMV promoters, TMV based vectors, CaMV based vectors, Potato-X-virus based vectors.

Unit V : Cloning and Expression: *Escherichia coli* expression vectors, promoters, plac, ptrp, pBAD, para, pgal, phage promoters, sp6, T3, and T7 promoters in addition to lambda pR, pL, pR' promoters for expression, Codon selection, maximizing expression, hybrid promoters (ptac, ptrc,), manipulation of clones gene to achieve expression, solubilization of proteins, fusion proteins (typically translational fusion vectors, but also cover transcriptional fusion strategy) and their applications.

Cloning in other Gram negative bacteria: Vectors derived from Inc Q replicon, IncP replicon, pBR1 to develop broad host range vectors, pSa replicon, shuttle between high copy to low copy *–in vivo procedure*.

Cloning in *Bacillus*: Transformation technique, plamids and vectors, expression vectors, shuttle vectors, pahge vectors, vectors favoring secretion of gene products, vectors for genome wide mutagenesis –insertion duplication strategy, vectors for generation of operon from dispersed genes on chromosome.

Cloning in *Stretomyces*: RSF1010 derivatives, plasmids with ref to their size, replication strategy, copy number and versatility (host range).

Cloning in *Yeast*: Shuttle vectors, replication origins, analysis of recombinant DNA,natural plasmids, integrative plasmid, centromeric plasmid, autonomously replicating –episomal vectors, with ref to structure, function and merti-demerit in types of cloning strategies, vectors for expression of recombinant in yeast.

Cloning in Archea.

References:

1. Genes by Benjamin Lewin Ed V, IX and X
2. Molecular biology by David freifelder
3. Molecular biology by Weaver

4. Molecular biology of the Gene by Watson and others
5. Recombinant DNA by J.D.Watson
6. Genetic Engineering by Nicoll
7. Manipulation and expression of recombinant DNA by Robertson
8. Genetics : Molecular Approach by T.A. Brown
9. Principles of gene manipulation and genomics by Twyman and Primrose 7th edition
10. Yeast Biotechnology by Berry
11. An introduction to genetic analysis by Griffith and others
12. Principles of Gene manipulation by Old and Primrose
13. From Genes to Clones –by Winnacker
14. Microbial Genetics by David freifelder

M. Sc. Biotechnology,

Paper XI

Developmental Biology

Unit-I: Basic concepts of development:

Potency- Totipotent pluripotent, multipotent, unipotent cells Commitment – Neuroblast multipotent neurons Differentiation –Specification : determination Autonomous specification and conditional specification.Syncytial specification, morphogenetic gradients, cell specification.

Primary germ layers: Ectoderm, Mesoderm, Endoderm, triploblastic and diploblastic animals.Fate maps and cell lineages

Genomic equivalence: Creation of sheep dolly as evidence for genomic equivalence

Imprinting: DNA methylation

Mutants, chimeras and transgenes for analysis of development (Fate mapping studies) Chick- quail experiment -GFP

Unit-II: Gametogenesis, fertilization and early development:

Production of gametes-Spermatogenesis and Oogenesis cell surface molecules in sperm-egg recognition in animals Ex. Sea urchins Bindin and EBR1(Bindin receptor).

embryo sac development and double fertilization in plants; zygote formation (introduction), cleavage, blastula formation in sea urchin, embryonic fields, gastrulation and formation of germ layers in animals- **Zebra fish**; embryogenesis, establishment of symmetry in plants; seed formation and germination.

Unit-III: Morphogenesis and organogenesis in animals:

Cell aggregation and differentiation in *Dictyosteliu discoideum*; axes and pattern formation in *Drosophila*, amphibia and chick; organogenesis – vulva formation in *Caenorhabditis elegans;* eye lens induction, limb development and regeneration in vertebrates; differentiation of neurons, post embryonic development-larval formation, metamorphosis; environmental regulation of normal development;

Sex determination - environment dependent in reptiles, location dependent

Unit-IV: Morphogenesis and organogenesis in plants:

Organization of shoot and root apical meristem; shoot and root development; leaf development and phyllotaxy; transition to flowering, floral meristems and floral development in *Arabidopsis* and *Antirrhinum.*

Unit-V: Programmed cell death, aging and senescence.

Programmed cell death-mechanism and significance

Genes and ageing

Insulin signaling cascade in c. elegans

Environmental and epigenetic causes of ageing

Plant senescence

References:

Developmental biology –By Scott Gilbert.

Aspects of floral development by Lein and others

Embryology of angiosperms by Bhojvani and Bhatnagar

Molecular cell biology by Lodish and others

M. Sc. Biotechnology,
Paper XII
Bioinstrumentation

UNIT I: Microscopic Techniques :

Properties of light, spectrum of light, Numerical Aperture,Types of lenses, magnification through a lens, Resolution limit & Resolving power of a lens.
Theory, Principle & Application of light microscopy: Bright field microscopy,Dark field microscopy,Phase contrast microscopy,Fluorescence microscopy,NIDC microscopy, Conofocal microscopy.

Principle,Types & Applications of Electron Microscopy : SEM, TEM, STEM, Different Fixation & Staining Techniques for EM,Freeze-etch & Freeze-fracture for EM, Image Processing Methods in Microscopy.

UNIT II :Separation Techniques:

Chromatographic Techniques: Theory, Principle & Applications of Paper,Thin-layer, Gel filtration, Ion exchange,Affinity,Reverse phase, Gas-Liquid & HPLC.(High performance liquid chromatography)

*ElectrophoreticTechniques:*Basic principles of electrophoresis, Theory & Applications of Paper, Starch gel, Agarose, Native and denaturing PAGE and Isoelectric focusing, 2D electrophoresis.

Unit III :Centrifugation :

Types of centrifuge machines, Preparative and Analytical Centrifuges, Differential centrifugation, Sedimentation velocity, Sedimentation equilibrium , Density gradient methods and their applications.

Unit IV : Biophysical Methods and Electrophysiological Methods:

Biophysical methods:

Analysis of biomolecules using UV-visible, IR Raman, Fluorescence, NMR ,CD-ORD, and ESR .Analysis using light scattering, Different types of mass spectrometry and surface plasma resonance methods.

Electrophysiological Methods:

Priciples and medical applications of

Single neuron recording, Patch-clamp recording, ECG, Brain activity recording, Lesion & Stimulation of brain, **Pharmacological Testing- PET,MRI, f MRI,CAT. ***

Unit V : Radio Labeling techniques:

Properties of different types of radio isotopes used in biology,Detection and Measurement of Radioactivity using Ionization Chamber, Proportional counter Geiger-Müller and Scintillation counters ,Incorporation of radioisotopes in biological tissues & cells, Autoradiography and its Applications,Safety guidelines.

References:

David Friefelder: physical biochestry- w. h. freeman and company

Wilson and walker:

Nath & Upadyay: Biophysical chemistry -himalaya

Gudeep and Chatwal :Instrumental methods of chemical -himalaya

Notes for teachers

* (Completion by Assignment & Test)

M. Sc. Biotechnology

Paper No. XIII 4-Credits

Industrial Technology

Unit I: Bioreactors :

Design of a basic fermenter: Design features, individual parts, baffles, impellers, foam separators, sparger, culture vessel, cooling and heating devices.

Calculations for designing a bioreactor.

Instrumentation and control of bioprocesses (Physical and chemical sensors for the medium and gases., online sensors for cell properties, off-line analytical methods Biosensors , computer control of fermentation process)

Different Bioreactor configurations: (Basic construction and types for distribution of gases)**:** Tube reactors, Packed bed reactors, Fluidized bed reactors, cyclone reactors, Trickle flow reactors,

Reactors for specialized applications: Alcohol fermentation

Unit II: Mass Transfer in reactors

Aeration /Agitation its importance; critical oxygen concentration;, mass transfer and diffusion, Gas - liquid exchange (two film theory for mass transfer) and O_2 transfer, derivation of equations for mass transfer in a fermentation media, determination of k_la , heat transfer (a brief account).

Media and air sterilization: introduction and the kinetics of death of microorganisms; batch and continuous sterilization of media, air sterilization, various type of sterilization equipments, sterilization of media by membrane filters, Sterilization of Bioreactors-*ex-situ* and *in-situ,* Merits and demerits of each

Scale up of Bioreactors

Unit III Fermentation process:

Development of Fermentation Process: Screening and Isolation of microorganisms, Strain improvement of the selected organisms ,designing of fermentation media for lab scale experiments, inoculum development and production; inoculum development strategies and procedure; storage of cultures for repeated fermentations, scaling up of process from shake flask to industrial fermentations (Economic considerations)

Growth of cultures in the fermenter: Kinetics of growth in Batch culture, continuous culture w.r.t. substrate utilization, specific growth rate, substrate utilization kinetics; steady state in a chemostat; Fed batch fermentations; yield of biomass, product; calculations for productivity,

Unit IV Industrial fermentation:

Study of industrial fermentation processes with respect to:

Microorganism, strain improvement (if any), media, production parameters, type of reactor and type process, down stream processing (involving separation ,purification packaging etc.)

Citric acid, penicillin, amylases, ethanol

Novel fermentation processes:Xanthan,PHB

Down stream processing :Separation of insoluble products (sedimentation, filtration, centrifugation, coagulation and flocculation) **Cell Disruption (**Mechanical methods, Non-mechanical methods) **Separation of soluble products(** Liquid-liquid extraction, aqueous two-phase extraction,

precipitation, adsorption, Dialysis, electro-dialysis, ultra-filtration and micro-filtration, cross-flow ultra-filtration and micro-filtration.)**Extraction** : Solvent extraction ,two phase liquid extraction, whole broth extraction Aqueous multiphase extraction. **Purification** – crystallization and significance

Unit V Biotransformation and Environmental Biotechnology

Introduction, types of reactions involved, procedures and applications with respect to steroids, antibiotics and pesticides transformations, Degradation of Xenobiotics from the environment Bioremediation

Microbial Leaching : Chemistry, organisms used, and applications

Biotechnology and waste management: Aerobic and anaerobic treatments,

Effluent treatment: Types, microbes used, Types of ETP plants

Bioinsecticides and Biofertilizers.

Bioterrorism.

Intellectual property and Ethical issues:

References:

1. Biochemical Engineering Fundementals,- Baily & Ollis Tata Mcgraw hill ,New york
2. Principles of Fermentation technology, -Stanbury & whittekar Butterworth-Heinemann.
3. , Biotechnology, A text book of Industrial Microbiology, Creuger & Creuger Sinaeur Associates
4. Biotechnology: A comprehensive treatise H.J.Rehm &Reed G,VCH
5. Industrial Microbiology -L.E.Cassida, Wiley Eastern
6. Biochemical Reactors Atkinson B Pion Ltd, London
7. Energetics of Microbial Growth, Battley, E.H. John Wiley &Sons
8. ,A.L Manual of Industrial Microbiology and Biotechnology 2nd Edition, Davies,J.E and Demain ASM, Publications
9. Metaboloic Engineering. Principles and Methodologies., Stephanopoulos,G.,Neilson,J, and Aristodou,A . Academic Press, San Diego
10. Encyclopedia of Bioprocess Technology : Fermentation,Biocatalysis, and Bioseperation ., Vol 5 Chisti Y John Wiley and sons, New York
11. Bioprocess Engineering Principles, Doran P.M.,-Academic Press, London
12. Basic Bioreactor Design. Van't Riet,K. and Tramper ,J. Marcel Dekker, New York
13. Seperation Process in Biotechnology, Asenjo, J.A., ed. Marcel Dekkar, New York
14. -Bioseperations : Downstream Processing for Biotechnology., Belter, P.A, Cussler, E.L.and Hu, W-S .John Wiley and Sons, New York
15. Basic Biotechnology, 2nd Ed. Colin Ratledge and Bjorn Kristiansen,Cambridge University Press.
16. . Biodegradation and Bioremediation. Alexander M Academic Press, SanDiego

M. Sc. Biotechnology

Paper No. XIV 4-Credits

Recombinant DNA Technology

Unit I Construction of Genomic Library and cDNA Library

Methods for generating fragments for library;

A- Restriction Endonuclease Methods –Complete digestion, Partial digestion, End filling, Removal of protruding termini, adding Restriction endonuclease site to the blunt fragments –Use of Linkers and Adaptors.

B- Mechanical shearing –generating blunt end creating sticky end with use of linkers and adaptors

C- Duplex cDNA synthesis/ generation of homopolymeric tail introduction of sticky termini

D- Direct Chemical synthesis

E- Polymerase Chain Reaction Method to generate Fragments

Methods for Sealing / Joining Strategies: homopolymeric tail, Sticky ligation, Linkers, Adaptors, TA cloining,

Introduction of Ligated Molecules into an appropriate host cell

A- Transfection –Phage based vectors

B- Transformation –plasmid based vectors

C- In Vitro Packaging, transduction with recombinant Phage or Cosmid

Selection and Screening Strategies:

Screening Methods –Direct and Indirect

A. Use of and examples of Markers,

B. Use of and examples of Reporters, Screening with gain of function (adding a new property to surrogate cell and exploiting this property directly for screening),

C. Indirection Methods –Hybridizations- Blotting –Southern, Northern, Western, South Western, Colony Hybridization, Plaque lift assay, North Western, Generation of probes –radioactive and non-radioactive, PCR mediated screening. Chromosome walk and Chromosome jump to obtain Contig or Chromosome alignment by hybridization strategy.

D. Immunological Screening - Ag-Ab mediated, South-Western and North-Western to detect clone having DNA –protein and RNA –protein interaction (binding sites).

Genomic Library: By λEMBL Vector, Maniatis's Strategy, Use of λDASH Vector and λFIX Vector, Genomic library with the use of Comid vector, PCR mediated genomic cloning, – functional cloning, Positional cloning or Marker Assisted Cloning.

cDNA library: Methods to obtain cDNA molecules:- Self priming, hairpin production, removal of hairpin by S1 Nuclease and addition of homopolymeric tail with the use of TDT at the end of first strand synthesis, directional cloning, use of random primer for second strand synthesis (nick translation), Non-directional cDNA cloning, Gubbar and Hoffman method, on plasmid cDNA synthesis

Full length cDNA Oligocapture method and Oligo capping method

Unit II Polymerase Chain Reaction (PCR) and Sequencing

Polymerase Chain Reaction (PCR)

A. PCR –Primer design, standard PCR/ optimization of PCR conditions, basic principle, enzymes used with respect to their properties, amplification on various types of Double Stranded template DNA.
B. Conventional PCR, Principle, Potential and Limitations
C. Types of PCR
    a. PCR with degenerate primer
    b. Amplification of an unknown target –inverse PCR
    c. Amplification assuming authenticity of amplified amplicon –Nested PCR,
    d. Combination of complementary oligostrands a specific primer to identify insertion of an unknown in a known DNA –Ligation Mediated PCR
    e. Introduction of small, insertion, small deletion, mismatches PCR for mutagenesis –site directed mutagenesis
    f. Error prone PCR –to identify catalytic domain, active site and essential region of an enzyme
    g. Quantification of mRNA –Reverse Transcriptase PCR, Real –Time RT PCR

Sequencing and In vitro synthesis

A. Chemical method of DNA sequencing –Maxam Gilbert's method
B. Enzymatic method – dideoxy method, Chain Termination method, Double Stranded DNA sequencing method by Sanger. Modification of Sangers method –Automated DNA sequencing,
C. Chemical Synthesis of DNA

Unit III Site Directed Mutagenesis and Genome mapping

A. Site Directed Mutagenesis:-

    a. Primer extension –with the use of single primer, double primer, doping primer method – making all possible amino acid substitutions
    b. Mutagenesis with the use of chemical modification of base –pyrimidine modification

c. PCR based site directed mutagenesis –random insertion of mutation throughout target gene
d. Phage display to facilitate selection of mutant peptides
e. Cell Surface display

B. Genome mapping

a. Restriction Fragment Length Polymorphism,
b. Singel Nucleotide Polymorphism –STS, EST, AFLP, FISH, GISH, Denatured gradient gel electrophoresis
c. Randomly Amplified Polymorphic DNA
d. Amplified Fragment Length Polymorphism
e. Use of Padlock's Probe

Unit IV Analysis of Transcriptome and Expression analysis of Proteins (Transcritpome and Proteome)

Reverse Northern Analysis, Macroarray, MPSS, SAGE, and DNA Microarray (principle, applications –comparative and constrast studies) means for global transcriptome

A. DNA Microarray:-Spotted DNA Microarray (Nylong macroarray, glass microarray), Oligonucleotide chips –in situ oliognucleotide synthesis (Photolithography and Affymetrix)

B. Proteome analysis:- Two-Dimensional Gel Electrophoresis (2-DGE) –tube gel experiment, limitations of proteome such as why it hasn't been developed as like DNA –Microarray, staining methods –traditional and improved with the reasons of improvement, improvement in gel resolution and preparation of protein extract for various different cell parts, use of solvents for solubilization of protein from different compartment, Multidimensional liquid chromatography, with mass spectrophotometry, MALDI-TOF, ICAT (specialized strategies used for protein quantification), Antibody array, Antigen array –used to measure antibodies in solution.

Unit V Applications of Recombinant DNA Technology

A. Protein engineering:- αAAT, Subtilisin, growth hormone engineering, biphasic antibody engineering

B. Metabolic Engineering:–Phenylalanine synthesis, Synthesis of Indigo dye, Vitamin C –Ascorbic Acid, Vitamin E synthesis.

C. Gene Silencing and Antisense technology:- Quenching, Co suppression, RNA interference, SiRNA, Rasi RNA, Micro-RNA various pathways/ strategies of inhibiting gene expression. **Ribozymes:-** Principle, Mechanism of action, role in gene silencing, Designing Ribozymes for commercial purposes.

References:

1. Principles of Gene Manipulations 5th Edition, –Old and Primrose
2. Principles of Gene Manipulations and Genomics, 7th Edition, Primrose and Twyman
3. Genes X –Benzamin Lewins
4. From Genes to Clones, Winnacker
5. Molecular Cloning : Volume I, II, and III –Russel *et al.*
6. Current Protocols in Molecular Biology
7. Molecular Biotechnology –Glick
8. Recombinant DNA Technology by Watson
9. PCR and Applications

M. Sc. Biotechnology

Paper No. XV 4-Credits

Tissue Technology

Unit I- Introduction to tissue culture

A Plant tissue culture:

Media composition: Theory of limiting factor with respective to inorganic salts (Major and minor elements) organic chemicals (carbohydrates, vitamins)

Growth regulators (Auxin, cytokinin, gibberellins, ascorbic acid, ethylene) Amino acids (L-amino acids and its role in Tissue culture) Antibiotics (Ampicillin, Carbenicillin, Cefotaxime, Gentamycin sulphate, methylol urea, Polymixin B Ribavirin, Streptomycin) Uses

Chemically undefined constituents and natural complexes (gelling agents, activated charcoal, casein HL, coconut milk, corn milk, corn starch, potato extract, yeast extract, antioxidants

Selection of media for specified application: Callus induction media, chlorate selection medium, Embryo culture medium, Green algae medium, Propogation medium

Standardization of media and glassware

Cellular Totipotency

Callus culture and dynamics of callus growth:

Organogenesis and its applications:

Somaclonal variation and its application-

Micropropogation and its application: MP through shoot and bud meristem Tip culture, in vitro tuberization, somatic embryogenesis

B. Animal Tissue culture:

Media, Natural, artificial, serum, serum free media, chemically defined media and protein free media

Primary culture, Tissue disaggregation, secondary culture, cell lines and maintenance, Assessment of growth, measurement of cell death

UNIT-II-Methods in Plant Tissue Culture

Organ culture: Root culture, shoot tip culture (meristem culture), flower bud culture, ovary culture, ovule culture, embryo culture, anther culture Callus culture and its dynamics Somatic embryogenesis and artificial seeds Protoplast culture, protoplast fusion and somatic hybridization –

Unit III Methods in Animal Tissue Culture

Monolayer culture: Roux bottle, roller bottle, multitray, synthetic hollow fiber, bead bed reactors, continuous flow cultures, Airlift fermentors

Immobilized cultures

Entrapment cultures, Porous carriers, Fixed bed (Parosphere and fluidized beds)

Unit IV Transformation methods

Direct gene transfer: PEG mediated transfer, microinjection, protoplast and intact cell electroporation and gene gun, chloroplast transformation.

Indirect method: Agrobacterium mediated gene transformation

Animal cell: DNA calcium phosphate coprecipitation method, Lipofection, electroporation, DEAE dextran method, Microinjection

Unit V Application of Cell Science

Plant cell culture: Production of biodegradable plastics, synthesis of primary and secondary metabolites with desirable properties. Bio-Pharmaceutical, edible vaccines, Bioremediation. Increased crop productivity by altering plant physiology, Enhanced nutrient utilization, tolerance to abiotic stress and improved disease resistance. Reduction in the use of harmful agrochemicals by increasing Herbicide resistance and Insect resistance.

Enhanced nutritional content: Golden Rice

Animal cell culture: Production of vaccines, interferons, antibiotics, embryonic stem cell, gene therapy, transgenic mice, cattles, sheep, fish. Tissue engineering,

Ethical issues

Practicals

1. Introduction to plant tissue culture.
2. Media preparation for plant , animal,and sterilization
3. -Induction of callus culture, regeneration from callus.
4. Anther culture
5. Preparation of artifical seed
6. Isolation of protoplast
7. Micropropagation
8. Agrobactrium mediated transformation
9. Gene transfer in plant cells,animal cells
10. Introduction toanimal culture.
11. Establishment of primary culture, secondary culure.
12. Preservation of cell lines.
13. Regenration of frozen cellsin vitro
14. Marker gene assay.

References

1. A text book of genetic engineering by Desmond Nicoll
2. Agrobacterium: From biology to Biotechnology by Tzvi Tzfira
3. Principles of gene manipulation by Primrose
4. Animal cell culture by Jon Masters
5. Plant cell and tissue culture – A tool in Biotechnology- by Ashwani Kumar
6. Laboratory manual of Plant Biotechnology By Purohit-2
7. Methods in Plant tissue culture By U Kumar-1
8. Plant tissue culture Bhojwani:
9. Introduction to Plant Tissue Culture Kalyan Kumar DE
10. Practical application of Plant Molecular Biology R.J.Henry,Chapman and Hall
11. Culture of Animal cells-3rd Edition Freshney Wiley liss
12. Mammalian Cell Biotechnologyy-Apractical Approach, M,Butler Oxford University press ,New York
13. Animal Cell culture techniques, Martin Springer

M. Sc. Biotechnology

Paper No. XVI 4-Credits

Introduction to Bioinformatics

Unit 1-Basics of Bioinformatics

Introduction to Bioinformatics, History of Bioinformatics, Scope and applications of Bioinformatics, Genome sequencing strategies: Whole genome shotgun sequencing and Hierarchical genome shotgun sequencing with reference to model organisms, Human Genome Project, Rough and final draft of HGP, Goals of Human Genome Project.

Unit 2-Biological Databases

Nature of biological data, Overview of Bioinformatics resources on the web-NCBI/EBI/SIB etc, Introduction to biological databases, Nucleic acid sequence database-GENBANK/EMBL/DDBJ, Protein sequence database; Primary protein sequence databases; Swiss_Prot, PIR, MIPS, NRL_3D, TrEMBEL, Secondary Protein Sequence Databases: PROSITE, PROFILE, BLOCKS, PRINTS, Pfam, IDENTIFY. Specialized database;-OMIM, Literature database-PUBMED, Structural databases; PDB, NDB, MMDB, Biological information search engine-Concept and Applications.

Unit 3- Sequence Analysis

Overview of concepts in sequence analysis, pairwise and multiple sequence alignment and their and significance, Local and global sequence alignment and its algorithm, Sequence similarity search tools- BLAST-Concept, algorithm and working procedure, FASTA, Multiple sequence alignment, ClustalW, **Phylogenetic tree** - construction methods, Phylogenetic analysis, Tree Evaluation method-Bootstrap.

Unit 4- Macromolecular structure prediction and Visualization

Protein structures; Primary, Secondary, Tertiary and Quaternary. Protein folding, Secondary structure prediction methods-Chou-Fasman, GOR, Jpred, Three-dimensional structure prediction Methods; Comparative modeling (Homology modeling), Fold recognition or Threading method, *Ab-Initio* methods, Molecular Visualization using Rasmol, Cn3D, Comparative Genomics and Proteomics.

Unit 5- Bioinformatics in the study of metabolic pathways

Metabolic Pathway databases-KEGG, EcoCyc and MetaCyc, Enzymes, Compounds and Reaction databases- LIGAND -Biochemical Compounds and Reactions, BRENDA - Comprehensive Enzyme Information System, Representation of Metabolic Pathways,

Comparison of Metabolic Pathways, Metabolic Pathways comparison tools.

Recommended books

1. Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins -By: Andreas D. Baxevanis (Ed), B. F. Francis Ouellette (Ed) Publisher: Wiley, John & Sons, Incorporated ISBN: 0471478784
2. Introduction to Bioinformatics- By: Arthur M. Lesk Publisher: Oxford University Press, ISBN: 0199251967
3. Bioinformatics and Functional Genomics-By Jonathan Pevsner Publisher: Wiley-Blackwell, ISBN: 0470085851
4. Protein- Protein Interaction-By C. Frieden & L.W. Nichol. Publisher: John Wiley & Sons, Chichester, UK.
5. Introduction to Bioinformatics, (Atwood, T. K. and Parry-Smith, D. J).
6. An introduction to Computational Biochemistry. (C. Stain Tsai, A. John Wiley and Sons, Inc., publications).
7. Bioinformatics; Methods and applications; Genomics, Proteomics and Drug Discovery; (Rastogi, S. C. and Mendiratta and Rastogi, P.

LC 09: Practicals based on theory paper IX

1. Purification of Immunoglobulin by Precipitation
2. Affinity Purification of Immunoglobulin
3. Preparation of Enzyme Conjugated Antibodies
4. Isolation of O & H Antigen from *Salmonella typh0069*
5. Diagnostic Assay for Typhoid using Widal Kit
6. Enzyme Linked Immunosorbent Assay
7. Diagnosis of RA by Agglutination
8. Titration of *E.coli* Phages
9. Determination of Burst Size of Phages
10. One Step Growth Curve for Determining Virus Titre
11. Viral DNA Extraction
12. Clinical Diagnosis of Viral Diseases by PCR
13. Isolation of Plant Viruses from Diseased Material

LC 10: Practicals based on theory paper X

1. Study of conjugation in *E.coli* and score for a marker
2. Generalized transduction in *E.coli* using P1 phag
3. Transposition of tn family and insertional inactivation in *E.coli*
4. Phage titration with P1 phage
5. Gene expression in *E.coli* and yeast-blue white
6. Isolation of plasmid from bacillus sp.
7. Plasmid restriction digestion (only linearization)
8. Transformation in *E.coli*.
9. Transformation in *Bacillus sp.*
10. Electrelution to purify the DNA

LC11: Prcaticals based on theory paper XI

1. Study of Egg(s). (Drosophila / Chicken).
2. Study of totipotency in plant (Growing a carrot plant from adult cells).
3. Study of cleavage pattern in chicken/snail (Discoidal/spiral).
4. Study of developmental stages in chick embryo.
5. Establishment of drosophila culture and its maintenance.
6. Study of metamorphosis in *Drosophila*.
7. Study of cell death during embryonic development in chick and its role in morphogenesis and histogenesis (by using Neutral Red/Nile blue/methylene blue).
8. Protein profiling of *Drosophila* larva at various stages of development.
9. Effect of temperature on heart of the chick embryo.
10. Study the effect of teratological substances on chick embryo development.

LC12: Practicals based on theory paper XII

1. Paper Chromatography of amino acids- Ascending and Descending methods.
2. Separation of sugars by chromatography.
3. TLC of lipids and sugars.
4. Column chromatography for proteins, pigments using sephadex G-50
5. Paper electrophoresis.
6. Agarose electrophoresis-separation of bromophnol blue and xylenecynol.
7. Determination of molecular weight by PAGE- native and SDS
8. Immunoelectrophoresis- serum proteins

LC13: Practicals based on theory paper XIII

1. Random and strategic screening for a metabolite (**screening for citric acid producing organisms)**
2. Screening, enrichment and isolation for a secondary metabolite producer from the environment **crowded plate technique for antibiotic producing organisms**
3. Determination of TDP & TDT of *E .coli* for designing of a sterilizer
4. Determination of Growth curve of yeast and compute growth rate & growth yield
5. Strain improvement of the industrially important isolate (*A.niger* / yeast) using EtBr for higher yield of the product
6. Media balancing experiments: carbon and nitrogen as variables (in alcohol fermentation)
7. Alchohol Fermentation: using different substrates and its downstream processing.
8. Production of Organic acid /s by fermentation
9. Antibiotic fermentations **Penicillin** (upto bioassay)
10. Microbial Enzyme production and its characterization-**Amylase**
11. Bioinsecticide / Biofertilizers: isolation production purification and assay
12. Microbial leaching-using Thiobacillus thiooxidanse (NCIM strain)
13. Effluent treatment – A]dye degradation by microbial cultures

    B] Reduction in COD/BOD physical, chemical and biological treatments

LC 14: Practicals based on theory paper XIV

1. Isolation of total genomic DNA –Bacterial
2. Isolation of Nuclei –yeast
3. Isolation and purification of yeast mRNA
4. Restriction digestion for mapping –pBR322 restriction map
5. Restriction and separation of restriction fragments of Bacterial chromosomal DNA
6. Restriction digestion, separation and transfer of Eukaryotic genomic DNA – Southern Blotting
7. Northern Blotting
8. Western Blotting
9. Preparation of single strands –physical and chemical methods
10. Cloning of a trait and selection of the same in *E. coli*,
11. Studying expression (qualitative and quantitative) of cloned trait in *E. coli*
12. DNA ligation –ligase reaction, testing efficiency of ligation.

LC15: Dissertation in lieu of two practical courses

01. Dissertation is being submitted in lieu of two laboratory courses **(Bioinformatics and Tissue Technology)**, weighing 50 marks (4 credits). Although this is submitted in lieu of stated courses, actual project may or may not be directly related to these two courses. Project should however, be directly related to any of the aspects of sixteen theory courses or remaining fourteen laboratory courses.

02. Dissertation writing should be as a manuscript submitted to "Cell", an international peer reviewed journal. Regional format would not be entertained. Mentor and student, both, are expected to understand the writing style of research paper (full length) published in Journal titled "Cell". The cell word should not be mistaken for cell biology books or any such standard or substandard books.

03. Dissertation would include abstract, introduction, materials and methods, results, discussion, acknowledgments, references in chronological order. The writing should not be less than 4000 words without space, excluding figures and tables.