Syllabus

B.Sc. IIIrd Year (Microbiology)

[ Semester-Vth & VIth ]

[ Effective from – June, 2011 & onwards ]
Unit -1:-

• DNA/RNA as genetic material,
• Molecular properties of DNA – DNA melting, breathing, bending, flexibility, Novel structure of DNA, Linking number, role of major & minor groves in protein binding..
• History of molecular biology and genetic engineering
• DNA as genetic material- experimental proof- Griffith’s experiment
  Hershey_Chase experiment
• RNA as genetic material – experimental proof
• DNA replication- models of replication, experimental evidence for semiconservative replication, enzymology of replication, mechanism of DNA replication
• Post replication modifications (methylation); role of restriction endonuclease, hsd, dam,dem system of methylation

Unit :2.

• Salient features of Genetic code
• Transcription-structure of RNA polymerase, mechanism of transcription, post transcriptional modification.
• Translation- activation of amino acids, charging of t- RNA, Ribosomes, m-RNA, t-RNA
  a) initiation
  b) elongation
  c) termination
• Regulation of gene expression at the level of transcription
  a) lac Operon

Bacterial Recombinations:
• Transformation-discovery, nature of transforming principle, competence factors, mechanism of transformation
• Conjugation-discovery, structure and properties of plasmids (F, R, Col., ) Plasmid incompatibility, process of conjugation-(F, HFr, and F mediated transfers)
• Transduction –mechanism of generalized and specialized transduction, Abortive transduction, lysogenic conversion.
Unit 3: Mutations

- Spontaneous mutations (replica plating, fluctuation test)
  Base pair substitutions and frame shift mutations.
  Mutagenesis by physical and chemical agents.
  UV rays, X-rays, Base analogues, Agents modifying purines and pyrimidines, Nitrous acid, DMS, EMS, EES. Agents producing distortions in DNA -- Proflavin, Acridine orange, Intercalating agents (Ethidium bromide).
- Suppressions – true reversion versus genetic suppression (intragenic and intergenic suppressions)
- Site directed mutagenesis

Paper XVIII: Microbial metabolism

Unit 1

- Enzymes: Definition, properties, specificity, active site, activation of enzymes, mechanism of action of enzymes (lock and key, induced fit, ping-pong)
- Nomenclature and classification of enzymes
- Factors affecting catalytic activity of enzymes (pH, temp., enzyme conc., substrate conc., metal ions, time)
- Michaelis-Menten equation: derivation and significance
- Types of enzymes: extracellular, intracellular, constitutive and inducible.
- Enzyme inhibition: Irreversible, reversible (competitive, uncompetitive, in competitive) and metabolic antagonism, feedback inhibition.
- Elementary knowledge and uses of isoenzymes.
- Immobilized enzymes and allosteric enzymes.
- Commercial uses of enzymes (any five) – (food, leather, textile, environment, pharmaceuticals and clinical)
- Types of co-enzymes (NAD, FAD, Lipoic acid, VitB12, Thiamine pyrophosphate) and reactions catalysed (atleast two)

Unit 2

Definitions: Metabolism, anabolism, catabolism, free energy.

- Bioenergetics: chemical links between catabolism and biosynthesis, energy coupling through ATP and through pyridine nucleotides, Central role of ATP-ADP system.
- Modes of energy yielding metabolism: Definition and features of fermentation, respiration and photosynthesis.
- Fermentation of carbohydrates:
  EMP, HMP, ED, Phospoketolase pathway (pentose, hexose).
  Alcoholic, homolactic, mixed acid, butanediol, butyric, acetone-butanol fermentations.
• Aerobic respirations:
  RETC : location functions, components, redox carriers, oxidative phosphorylation
  artificial electron acceptors, bacterial cytochrome systems
• TCA cycle, glyoxylate cycle, anaplerotic sequences, regulation of TCA.

Unit : 3 (15)
• Catabolism of saturated (16 carbon) and unsaturated fatty acids (16 carbon) by β oxidation
• Degradation of proteins and amino acids : proteolysis, putrefaction.
• Transformation of aminoacids : oxidation, reduction, decarboxylation, deamination . (one example of each).
• Nucleic acid catabolism: DNA, RNA depolymerization, degradation of nitrogenous bases
  (mention end products without pathway)
• Biosynthesis of nucleotides: Purine and pyrimidine nucleotides, conversion of ribonucleotides to deoxyribonucleotides.
• Carbohydrate synthesis : peptidoglycan.

Practical paper XIX
1) Determination of one step growth curve of bacteriophage.
2) Replica plating for isolation of streptomycin resistance spontaneous mutant of E. coli.
3) Isolation of lac mutants of E.coli. ( Lac ) by UV induced mutagenesis and chemical mutagens.
4) UV damage and photoreactivation.
5) Study of transformation in E. Coli.,
   i) Preparation of competent E. Coli.
   ii) Enumeration of transformed cells.
   iii) Determination of plasmid transfer efficiency.
6) Study of conjugation in E.Coli. (plate method.)
7) Demonstration : Polymerase chain Reaction ( PCR )
Practical Paper XX

1) Preparation of buffers and reagents.

2) Study of enzymes :- α-amylase, caseinase, catalase, deaminase, desulfurase, gelatinase, lecithinase, oxidase.

3) Effect of pH, temp, substrate concentration on α-amylase activity.

4) Demonstration of nitrate reduction

5) Demonstration of decarboxylation of amino acid.

6) Isolation of photosynthetic bacteria by column method

7) Primary screening for :
   i) Starch hydrolyzers.
   ii) Organic acid producers.
   iii) Antibiotic producers.
B.Sc. III Year [Semester-VI]

Paper- XXI Recombinant DNA Technology

Unit 1
(15)
- Recombinant DNA technology : definition, tools used for cloning, restriction endonucleases (types, nomenclature, recognition sequences, with examples).
- Modification of blunt ended DNA (T4 ligase, homopolymer tailing, linkers and adapters)
- Vectors : properties of good vector, cloning and expression vectors. (pBR322, pUC8, pSC101,) , Bacteriophage vectors (λ phage, M13 phage vectors), phagemid, cosmids, YAC / MAC.
- Genetic engineering – principles, cloning organisms, uptake of DNA (Calcium chloride treatment, electroporation, protoplast fusion, liposome), selection of recombinant clones.
- Genomic library (construction and identification of desired clone)

Unit 2
(15)
- Nucleic acid & protein blotting techniques : Southern blotting, western blotting, northern blotting.
- Colony hybridization
- DNA sequencing (Maxam & Gilbert)
- Probes (preparation & labeling), its uses
- PCR

Unit 3
(15)
- Gene therapy
- Applications of genetic engineering
  a) Agriculture
  b) Human and animal health
  c) Industries
  d) Environment
- Ethical issues of genetic engineering
- Transposition- Discovery, structure and types of bacterial transposons, mechanism of transposition, spread of antibiotic resistance, mutation due to transposition
Paper – XXII Industrial Microbiology

Unit 1. (15)

- Design of typical fermenter, types of fermenters (Single, multiple, recycle, airlift)
- Screening methods: primary, secondary.
- Strain improvement methods, increasing product yield.
- Preservation methods (lyophilization, freezing, mineral oil, soil stocks)
- Inoculum development
- Fermentation media: raw materials, media formulation, pretreatment, sterilization, contamination and its control, inoculum media, buffers, antifoam agents and precursors.
- Scale up of fermentation
- Phage contamination and control
- Down stream processing

Unit 2 (15)

- Antibiotic fermentations: Penicillin.
- Vitamin fermentation: Vit. B-12
- Amino acid fermentation: L-lysine (direct and indirect)
- Organic Solvent: Ethyl alcohol fermentation
- Organic acid fermentation: Citric acid

Unit 3 (15)

- Enzymes- α – amylase (bacterial & fungal)
- Bakers yeast production
- Vaccines: Genetic recombinant vaccines
- Biofertilizers – (Azo, Rhizo, and PSB)
- CH₄ fermentation
- Biopesticide production
B.Sc III year practical
Practical paper XXIII

1) Isolation of genomic DNA from *E. coli*.
   i) Purification of DNA by phenol extraction method.
   ii) Concentration of DNA by ethanol precipitation.
   iii) Separation of DNA using agarose gel electrophoresis.

2) Restriction analysis of *E. coli*.

3) Isolation of *E. coli* plasmid DNA

4) Separation of plasmid DNA by agarose gel electrophoresis.

5) Western blotting

6) SDS PAGE

7) Measurement of β-galactosidase activity using ONPG.
B.Sc III year practical
Practical paper XXIV

1) Production, detection and estimation of:

--------- Ethanol using *S. cerevisiae* var, ellipsoideus.
--------- Glutamic acid by *Micrococcus glutamicus*
--------- Citric acid by *Aspergillus* spp.
--------- α-amylase using *Aspergillus sp/Bacillus sp.*
--------- Penicillin by *Pencillium* spp.
--------- Biosurfactants using *Pseudomonas* spp.

2) Strain improvement (Physical/chemical agents) for α-amylase production using *Aspergillus sp/Bacillus sp.*


4) Separation of proteins using agarose gel electrophoresis.

5) Bioassay of Penicillin/ Vit B12

6) Study tour and report presentation.
Reference books for B.Sc.III year

1. A.H. Rose: *Chemical Microbiology* - An introduction to Microbial Physiology, Butterworth World student, LONDON.
2. Campbell Peter N. & Smith Anthony D.: Biochemistry illustrated, Churchill Livingstone, NEW YORK.
5. Lehninger Albert L.: Biochemistry, Kalyani Publishers, NEW DELHI.
11. A.H. Patel: Industrial Microbiology, McMillan(India) Ltd., BOMBAY.
12. Casida L.E.: Industrial Microbiology, Willey Estern Ltd., NEW DELHI.
13. Prescott & Dunn: Industrial Microbiology, MacGrow Hill Co. Ltd.
18. Tikekar P.G.: Practical Biochemistry for Medical Students, Purvi Pustak Kendra, BOMBAY.
19. Avinash & Kakoli Upadhay: MOLBIO, Himalaya Publications
24. Joshi P.: Genetic engineering & its applications, Agrobious, JODHPUR (India).
25. Nilima Rajvaidya & D. Markendey: Genetical and Biochemical applications of Microbiology, APH Publishing Co. NEW DELHI.
27. Strickberger M.: Genetics, Prentice Hall of India Pvt. Ltd., NEW DELHI.

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