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S.P. Mandali's

RAMNARAIN RUIA AUTONOMOUS COLLEGE

Syllabus for: **M.Sc. Part II**

Semester III

Program: M.Sc. Life Science

Course Code: LIFE SCIENCE (RPSLSc)

(Semester based credit and grading system with effect from academic year 2017-2018)

M.Sc. Part - II Life Sciences - Biotechnology Syllabus

Choice based Credit and Grading System
The Academic year 2019-2020

SEMESTER III

COURSE CODE	UNIT	TOPIC HEADINGS	CREDITS	L / WEEK
Paper I	**Genetic Engineering**			
RPSLSc 301	I	Recombinant Techniques	4	4
	II	Microbial Cell Factories		4
	III	Engineering Lower eukaryotes I		4
	IV	Engineering Lower eukaryotes II		4
Paper II	**Fermentation Technology**			
RPSLSc 302	I	Upstream Processes	4	4
	II	Fermentation process I		4
	III	Fermentation process II		4
	IV	Downstream Processes		4
Paper III	**Industrial Enzymes, Tissue Culture and its applications**			
RPSLSc 303	I	Enzymes in Industry	4	4
	II	Plant Tissue Culture		4
	III	Animal Tissue culture		4
	IV	Assisted Therapies		4
Paper IV	**Research Methodology and Quality Control**			
RPSLSc 304	I	Research Methodology	4	4
	II	Scientific writing		4
	III	ISO, GMP, GLP		4
	IV	Using Computers in Research		4

SEMESTER III

PAPER - RPSLSc 301

Genetic Engineering

Unit I: Recombinant Techniques (15L)
Introduction to recombinant proteins.
Modifying genes/regulating sequences/proteins: Site-directed Mutagenesis Methods: Error prone PCR, Cassette mutagenesis, Site Saturation mutagenesis, Overlap PCR, DNA/Domain/Exon shuffling, ICTHY, SCRATCHY, RACHITT.
Expression: phage, cell, DNA, RNA, ribosome and IVC display,
Analysis and detection, applications - modifying activity, substrate specificity, cofactor requirement, increasing stability, pH and temperature optima,
Construction of deregulated mutants resistant to feedback inhibition and repression: Examples of modified proteins.
Genome editing: Homologus recombination, zinc finger nuclease, TALENS, CRISPR/Cas9, Modified nucleases – meganuclease.
Application of RNAi in strain improvement: use of siRNA, shRNA, miRNA, ribozymes and riboswitches to regulate and optimize gene expression.
Metabolic Engineering: Metabolic pathway analysis and modelling – approaches, Methods for metabolic engineering, Model organisms – *E. coli*, *B. subtilis*, *Saccharomyces*, plants and animals, Industrial applications.
Systems Biology and Synthetic Biology for strain improvement: Omics analysis, *in silico* modelling, development of improved strains.

Unit II: Microbial Expression Systems (15L)

Prokaryotic: *E. coli*: Expression systems – pET, pBAD, λPL, prhaBAD systems, Expression of Foreign Genes in Bacteria – Problems, optimization of expression: host, transcriptional, translational, post translational compatibility, solubility and purification, transport and localization (use of Promoters, Ribosome Binding Site, Fusion Proteins, signal sequences, Tags and cleavage sites), Modification of gene – codon optimization, host strain modification Expression of Native Proteins, Detecting Expression of Foreign Genes.

Gram Positive Bacteria: *Bacillus subtilis*, *Lactobacilli*, *Streptomyces* – Expression systems, optimization of expression and applications.

Unit III: Engineering Lower eukaryotes I (15L)

Algae: Types, Culture systems, Genetic modification - transformation strategies, selection markers, promoters, terminators, translational regulation of protein production, strategies for efficient protein production, applications – increasing photosynthetic efficiency, yield of commercial and therapeutic products, Risks of GM algae.

Filamentous fungi – Host strains, transformation strategies, selection markers, promoters, terminators, translational regulation of protein production, strategies for efficient production, signal sequences, gene fusion approach, overproduction of foldases and chaperones, role of glycosylation, heterologous and homologous gene expression, humanization of filamentous fungi (*Aspergillus*, etc.), applications - pharmaceutically important secondary metabolites, medicinal mushrooms (*Ganoderma*, etc.), polysaccharides from basidiomycetes for immunostimulating and anticancerous activity.

Unit IV: Engineering Lower eukaryotes II (15L)

Yeasts: Yeast Selectable Markers and Vector Systems, commercially used yeast strains (***S.*** *cerevisiae* and *Pichia*) and their expression systems

Heterologous Protein Production - Design parameters: Source of DNA, Heterologous mRNA and protein levels and downstream applications, humanization of yeast for post translational compatibility.

Uses: Analysis of Genes, Genomes and Protein-Protein Interactions - YAC Technology, Constructing Gene Knockouts and Novel Reporter Systems, synthesis of commercially compounds.

Protozoa: Advantages of protozoan expression systems from *Dictyostelium discoideum*, *Leishmania tarentolae*, *Perkinsus marinus and Tetrahymena thermophila*, cultivation and applications of protozoan biotechnology.

PRACTICALS – RPSLScP 301:

1. Isolation of plasmid DNA from *E. coli*.
2. Transformation of *E .coli* and blue-white colony screening.
3. Isolation of Protease degraders from soil and estimation of the protease activity.
4. Preparation and regeneration of fungal protoplast.
5. Detection and estimation of gene copy number by real time PCR (demonstration).
6. Transformation of Yeast
7. Slide culture of filamentous fungi with nuclei tracking using DAPI stain.
8. Replica Plating.
9. Restriction Fragment Length Polymorphism (RFLP).

References:

1. Molecular Biology and Biotechnology, 5th and 4th edition by J. M. Walker and R. Rapley
2. Biotechnology, Concepts and Applications by R. R. Vittal and R. Bhat
3. Biotechnology, Principles and Applications by S. C. Rastogi More Gene Manipulations in Fungi by J. W. Bennette and Linda L. Lasure
4. Microbial Metabolism and biotechnology, ebook by Horst Doelle

5. The Metabolic Pathway Engineering Handbook- Fundamenals Christina D Somlke
6. Systems Biotechnology for strain improvement. Trends in Biotechnology. Volume 3 (7), 2006.
7. Molecular Biology: A laboratory Manual, 2nd edition, 1989: Maniatis, Fritsch and Sambrook
8. Molecular Biology: A laboratory Manual, 4th edition, 2012: M. Green and J. Sambrook

PAPER – RPSLSc 302

Fermentation Technology

Unit I: Upstream Processes (15L)

Fermenter design: Components of the fermenter, sterilization, aeration and agitation.
Types of Fermenters: batch, continuous, air lift, fluidized bed, stirred tank.
Isolation and Screening of microorganisms: Isolation of microorganisms from various sources, Preservation, Primary and Secondary Screening of microorganisms.
Fermentation Media: Definition, Criteria, Various components, Types: crude and synthetic, sterilization, rheology of various components of media.
Microbial growth: General parameters, growth kinetics for various fermentation and types of stock culture, scaling up of culture for fermentation.
Process improvement in Fermentation: Improve strains, media, pH, temperature, aeration and agitation.

Unit II: Fermentation process I (15L)

Single Cell Protein, Biomass and Immobilization: Need of single cell production, production of bacteria, yeast, algae, fungi. Immobilization: cells and enzymes, methods of immobilization, applications.
Commercial Fermentations: Cheese: Culture, Fermentation process, Applications.
Alcohol: Wine, Commercial Ethanol (by-product fusel oils): Culture, Process and Applications.
Acids: Lactic acid industrial production and applications.
Carbohydrate: High fructose corn syrup.
Flavour/fragrance production with example.

Unit III: Fermentation Process II (15L)

Biotransformations: Classification and charactristics of enzymes – OTHLIL, applications of enzymes: (chiral synthesis of enantiomerically pure compounds, resolution of isomers). Examples of biotransformations: Oxidoreductases- Oxidation of 1- amino - D - sorbitol in the production of miglitol using *Gluconobacter oxydans* ; Hydrolases: any one example. Catalytic antibodies.
Secondary metabolites production from plants: Secondary metabolite types (alkaloids, terpenes [include IPP synthesis: Classic pathway and Alternate pathway for IPP synthesis in plastids], tannins, lignans pigments, lipids); Selection of callus cultures.
Examples of secondary metabolite production (industrial scale**)**: [shikonin, taxol (biosynthesis and bioreactor production) capsasin/ berbrine].
From microbes: Polymers [dextrans, xanthan gums, alginate], antibiotics [peptide, lantibiotics, aminoglycosides, beta lactam], cyclosporins, biosurfactants.

Unit IV: Downstream Processes (15L)

Product recovery: Product: internal, external, cell disruption methods: physical, chemical and biological, precipitation, filtration, centrifugation, extraction and purification, drying.
Product Economics: Microbial culture, Fermentation: Upstream and Downstream processes, recovery process, product processing.
Effluent Treatment: Need, Traditional methods disposal and disadvantage, physical, chemical and biological methods.

PRACTICALS: RPSLScP 302

1. Immobilization of cells.
2. Demonstration of fermenter/ chemostat.
3. Estimation of alcohol production: Sucrose/ fruit (s)/ sugarcane juice.
4. Isolation of cellulase producing microorganisms from natural source(s).
5. Determination of cellulose activity using Filter paper assay/ carboxy-methyl cellulose assay.
6. Secondary metabolite production using plant tissue culture (dye/ drug Alkaloids etc.)
7. Effect of elicitor(s) on the production of the plant secondary metabolite.
8. Estimation of tannins using the Vanillin Hydrochloride method.

Reference:

1. Principles of Fermentation Technology by Stanbury and Whitaker
2. Industrial Microbiology by Casida
3. Industrial Microbiology by Prescott and Dunn
4. Industrial Biotrasformations by A. Liese, K. Seelbach and C. Wandrey; Wiley – VCH
5. Role of Biotechnology in Medicinal and Aromatics Plants by Khan and Khanum Vol.1
6. Plant Tissue Culture by M. K. Razdan

PAPER – RPSLSc 303

Industrial Enzymes, Tissue Culture and its applications

Unit I: Enzymes in Industry (15L)

Industries: Textile Processing, Leather Processing, Paper & Pulp Processing, Detergents and laundry.
Food biotechnology: Fruit and vegetable processing: juices, nectars, purees; syrup and glucose isomerases, enzymatic synthesis of aspartame.
Other industrial uses: Vinegar, Baking, Cocoa fermentation, Olive oil production, fish processing industries.
Nutraceuticals: Probiotics: lycopene, isoflavonoids, glucosamine, phytosterol.
Feed Biotechnology: lignocelluloses into feed using cellulases, silage.
Bio preservation: chemical preservatives and their safety concerns, LAB Bacteriocins. Types of bacteriocins, mode of action, applications and regulations.

Unit II: Plant Tissue Culture (15L)

Basics of plant tissue culture: totipotency, macro and micro nutrients, media.
Culture: Micropropagation, Callus culture, Somaclonal variation, Suspension cell culture, Protoplast culture, Somatic hybridization, Cybrids, Somatic embryogenesis and synthetic seed production.
Conservation: Improvement, exploitation and conservation of genetic resources, Cryopreservation of genetic resources.
Recombinant technology: Plant transformation by *Agrobacterium tumfaciens* [including mechanism of T DNA transfer in wild type Agrobacterium], *A. rhizogenes* its plasmid, Biolistic: factors that influence transformation success, chloroplast transformation: vectors, advantages and disadvantages of the technique.
Applications of transgenic: Overview, Recombinant proteins of pharmaceutical importance in plants including vaccine subunits, edible vaccines, from hairy root cultures.
Transgenic plants: Strategies for virus resistance, Herbicide resistance, Insect resistance, nematode infections and resistance, stress resistance [salt, water and temperature], Improved nutrition [carbohydrate, protein], improved shelf life; Novel applications: change in lipid profile for industrial purpose, biodegradable plastics, novel horticultural traits [flower colour, variegation].

Unit III: Animal Tissue culture (15L)

Basics of animal tissue culture: Methods of cell dissociation/separation and preparation of primary cell culture, characteristics of cells *in vitro*, cell culture growth parameters, detection, prevention and determination of contamination in tissue culture.
Culture: Primary cell culture, immortalized cell culture, stem cell culture and transformed cell culture. Specialized cells: bone marrow, myogenesis, skin cell culture, erythrogenesis-, chondriogenesis- *in vitro*, **Preservation:** Cryopreservation of tissues and cell lines.

Analysis and Production: cell synchronization, cell transformation *in vitro*, Mass cultivation-cytodex and biofermentors.
Applications: Stem cells & therapeutic cloning, Tissue engineering and 3D printing.

Unit IV: Assisted Therapies (15L)

In Vitro Fertilization (IVF): History, Causes of infertility, Stimulation protocols for IVF, Baseline assessment, sperm and egg culture, Preimplantation Genetic Screening/Diagnosis (PGS/D), Mitochondria replacement therapy (MRT), fertilization using ICSI, embryonic culture at various stages of development, Grading embryos, transfer of embryos- Direct embryo transfer, Zygote intrafallopian transfer (ZIFT), Assisted Zona Hatching (AZH); Risks of IVF.
Antisense therapy: Introduction, strategies. oligodeoxyribonucleotide, catalytic antisense RNA, triple - helix forming oligonucleotides (TFOs), production, and limitations, first generation antisense drugs, second generation antisense drugs.
Applications: cancer therapy, viral diseases, gene function analysis and in agriculture.
Gene therapy: Overview, viral and non-viral Vectors for somatic cell gene therapy, Gene therapy for inherited immunodeficiency syndromes, Cystic fibrosis gene therapy, HIV-1 gene therapy.

PRACTICALS: RPSLScP 303:

1. Isolation and partial purification of Acid/ Alkaline phosphatase from potato
2. Analysis of purification fold of the extracted enzyme
3. Determination of molecular weight of enzyme by SDS-PAGE
4. Isolation and estimation of Nutraceuticals (lycopene/ isoflavanoids) by TLC
5. Micropropagation of selected ex-plants.
6. Production of artificial seeds.
7. Preparation of plant protoplasts.
8. Transformation using *Agrobacterium* spp**.**
9. RAPD analysis (plants/ bacteria).
10. Establishment of Primary Culture (ATC) using a suitable source.

References:

1. Principles of Biochemistry by Lehninger, Nelson and Cox
2. Industrial Biotransformations by A. Liese, K. Seelbach and C. Wandrey; Wiley
3. Introduction to plant tissue culture by M. K. Razdan
4. Animal Cell Culture by Ian Freshney
5. Basic Cell Culture by J. M. Davis
6. A Textbook of In Vitro Fertilization and Assisted Reproduction by Peter R. Brinsden (2005)
7. In-Vitro Fertilization, Third Edition by Kay Elder, Brian Dale - (2011, Cambridge University Press)

RPSLSc 304

Research Methodology and Quality Control

Unit I: Research Methodology (15L)

Introduction: Meaning, Objectives and Motivation in research;
Types of research – Descriptive, Analytical, Applied, Fundamental, Quantitative, Qualitative, Conceptual, Empirical and Other Types of Research;
Research Approaches; Research Methods vs. Methodology; Research and Scientific Method;
Research Process: Steps of research process; Criteria of Good Research; Sampling, Sample size determination, Plan for data collection, Methods of data collection, Plan for data processing and analysis;
Scientific misconduct: Plagiarism, Fabrication, Authorship conflicts, Salami and imalas publication.

Unit II Scientific writing (15L)

Introduction: Meaning of Scientific and non-scientific writing; Scientific Vocabulary and grammar. Synopsis, Dissertations, Thesis, Posters.
Correspondence: Formal letters, cover letters, drafting emails, replying to reviewers.
Writing a Research paper: Title, Abstract, Introduction, Review of literature, Methodology, Observations, Results, Discussions, Summary, Conclusion, and Bibliography (Referencing and citation styles). Supplementary data.
Writing a Research Grant Proposal: Funding agencies, guidelines, structure of research proposals – Setting a budget (Manpower, Consumables, Equipment, Travel, Contingencies, Overheads) with justifications, Expected outcomes, Cost benefit analysis, Work plan, and Time schedule of activities.
Presentations: Presenting numerical data - Graphical, Tabular, Animations, Slides etc.

Unit III: ISO, GMP, GLP (15L)

Introduction: Over View of standards in ISO9000 Family
Key principles: Key principles of ISO 9000- Quality Management System
ISO 9001: Detailed study on ISO 9001:2015 standard, based on a seven principles of quality management, including a strong customer focus, the motivation and implication of top management, the process approach and continual improvement
Application: Sector specific Application of ISO 9001- Quality Management System adapted by various industries
Introduction to GMP (Good Manufacturing Practices) and GLP (good Laboratory Practices) in Pharmaceutical Industries.
Overview: of GMPs are enforcement by the U.S. Food Drug Administration (US FDA) under Title 21 CFR. Documentation requirement related to GMP and GLP.
Case studies for SOP preparation and CAPA (Corrective action Preventive Action).

Unit IV: Use of Software in Research (15L)

Literature Search: Query formulation, PubMed, NCBI, Google Scholar, Unpaywal, Shodganga.
Word Processing systems, LATEX.
Image Editors: Adobe Photoshop, Pagemaker.
Referencing: Mendeley, EndNote, Zotero.
Graphing and Statistics: Microsoft Excel, Google Sheets, GraphPad Prism, Max Stat, SPSS.
Antiplagiarism: Plagiarism checker X, TurnItIn, Online plagiarism checkers.
Presentation and Design: Microsoft PowerPoint, Google Slides, Microsoft Publisher.
Using Cloud tools, Microsoft One Note.

PRACTICALS: RPSLScP 304:

Literature review

References:

1. Research Methodology in the Medical and Biological Sciences by Petter Laake, Haakon Breien Benestad, Bjorn Reino Olsen (2007, Elsevier_AP)
2. Research Methodology - Methods and Techniques by C.R. Kothari (1985, New Age Publications)
3. The Oxford Book of Modern Science Writing (Oxford Landmark Science) 2009 by Richard Dawkins (Author, Editor)
4. Writing Science: How to Write Papers That Get Cited and Proposals That Get Funded (2012) by Joshua Schimel (Author)
5. The Best of the Best of American Science Writing (The Best American Science Writing) 2010 by Jesse Cohen (Author)
6. From Research to Manuscript A Guide to Scientific Writing (Second Edition) By Katz**,** Michael J. (Springer Publication)
7. Science Research Writing for Non-Native Speakers of English by Hilary Glasman-Deal (Author), Imperial College Press, London, UK
8. Scientific Writing and Communication by Angellka Hofmann, Oxford University Press (2014)
9. ISO 9000 quality systems handbook fourth edition by David Hoyle
10. International standard ISO 9001: quality management systems requirements 5th edition 2015-09-15.
11. Jürg P. Seiler - Good Laboratory Practice - the Why and the How (2005, Springer)
12. Good Manufacturing Practices and Inspection volume 2 (2007, World Health Organization)
13. GLP Essentials - A Concise Guide to Good Laboratory Practice by Milton A. Anderson - (2002, CRC Press)