

Advance Master and Ph D Degree Course
Course Title: 'Metagenomics and Genetic Engineering' Credit 4 (3 + 1)

Faculty member: Prof B. K. Konwar, Molecular Biology and Biotechnology

Theory

01. (a) Microbial communities: (i) Soil habitat, (ii) Un-culturable microorganisms, and (iii) Culturable microorganisms
(b) Microbial species for beneficial products and processes
02. (a) Metagenomics principles, practices and approaches
(b) Soil, water, plant and animal metagenomics
(c) Metagenomics for desirable genes
(d) Metagenomics tools for novel compounds
03. Prospect of metagenomics (mg) in (a) agriculture, (b) environmental remediation, (c) clinical science, (d) quorum sensing, (e) life sciences, (f) industry, (g) renewable energy
04. Metagenomic library construction and analysis of mg library including functional screening
05. Approaches to metagenomics (i) Sequence based analysis and (ii) Function based analysis
06. Methods for accessing soil and other metagenomes
07. Expression vector/host systems
08. Restriction digestion of mgDNA, extraction of DNA fragments from gel (by QIA quick gel extraction kit), isolation of expression vector (pET-32a) plasmid (from *E. Coli*), restriction digestion of vector plasmid, ligation, transformation
09. Isolation and restriction digestion of recombinant plasmid
10. Sequencing of the cloned DNA, sequence analysis and phylogenetic tree construction
11. Analysis of the cloned enzyme gene: (a) Phylogeny, (b) Protein sequence, and (c) Multiple sequence alignment
12. Expression study
13. Purification and optimization of recombinant protein: (i) SDS polyacrylamide gel electrophoresis, (ii) determination of active lipase using zymographic study (iii) quantification of total protein
14. Determination of activity of purified protein: (i) homology model and validation for protein structure prediction, (ii) biochemical characterization of purified protein, and (iii) dose dependent enzyme activity

15. Substrate specificity and effect of substrate concentration
16. Effect of various factors on the enzyme activity
17. Thermal stability study

Practical ‘Metagenomics for industrial enzyme lipase’

01. Soil sample collection from suitable sites and isolation of mgDNA, purification, restriction digestion, gel electrophoresis and elution of desirable fractions.
02. Multiplication of non-recombinant pET-32a plasmid harbouring bacteria.
03. Isolation and purification of pET-32a plasmid, quality and size determination through gel electrophoresis.
04. Cloning of mgDNA fragments into pET-32a
05. Multiplication of *E. coli* BL21 (DE3) competent cells
06. Transformation of *E. coli* BL21 (DE3) competent cells with recombinant plasmid
07. Analysis of transformation frequency using non-recombinant (pET-32a) plasmid
08. Functional screening of mglibrary for the lipase gene
09. Isolation and restriction digestion of recombinant plasmid
10. Sequencing of the cloned DNA
11. Expression study: purification and optimization of recombinant protein: (i) SDS polyacrylamide gel electrophoresis, (ii) determination of active lipase using zymographic study and (iii) quantification of total protein
12. Substrate specificity: effect of (a) substrate concentration, (ii) surfactants, (iii) metal ions, (iv) chelating and emulsifying agent, (v) common salt, and (v) organic solvents

Reference books

01. Metagenomics: Theory, Methods and Applications, Pub. Caister Academic Press, Ed. Diana Marco *Universidad Nacional de Cordoba, Argentina* Publication 2010, ISBN: 978-1-904455-54-7
02. Metagenomics: Current Innovations and Future Trends, Pub. Caister Academic Press Ed. Diana Marco *Microbiology Department, Estación Experimental del Zaidín (CSIC), Granada, Spain*, Publication 2011, ISBN: 978-1-904455-87-5
03. Metagenomics: Methods and Protocols, EdS. Streit, Wolfgang, Daniel, Rolf, Pub. 2010, ISBN 978-1-60761-823-2

Lecture Plan

Course Title: ‘Metagenomics and Genetic Engineering’ Credit 4 (3 + 1)

Faculty member: Prof. B. K. Konwar, Molecular Biology and Biotechnology

S.No	Topic	Lectures
01(a)	Microbial communities: (i) Soil habitat, (ii) Un-culturable microorganisms, and (iii) Culturable microorganisms	02
(b)	Microbial species for beneficial products and processes	01
02(a)	Metagenomics principles, practices and approaches	02
(b)	Soil, water, plant and animal metagenomics	02
(c)	Metagenomics for desirable genes	02
(d)	Metagenomics tools for novel compounds	02
03	Prospect of metagenomics (mg) in (a) agriculture, (b) environmental remediation, (c) clinical science, (d) quorum sensing, (e) life sciences, (f) industry, (g) renewable energy	03
04	Metagenomic library construction and analysis of mg library including functional screening	02
05	Approaches to metagenomics (i) Sequence based analysis and (ii) Functional analysis	02
06	Methods for accessing soil and other metagenomes	02
07	Expression vector/host systems	01
08	Restriction digestion of mgDNA, extraction of DNA fragments from gel (by QIA quick gel extraction kit), isolation of expression vector (pET-32a) plasmid (from <i>E. Coli</i>), restriction digestion of vector plasmid, ligation, transformation	03
09	Isolation and restriction digestion of recombinant plasmid	01
10	Sequencing of the cloned DNA, sequence analysis and phylogenetic tree construction	02
11	Analysis of the cloned enzyme gene: (a) Phylogeny, (b) Protein sequence, and (c) Multiple sequence alignment	02
12	Expression study	01
13	Purification and optimization of recombinant protein: (i) SDS polyacrylamide gel electrophoresis, (ii) determination of active lipase using zymographic study, and (iii) quantification of total protein	03
14	Determination of activity of purified protein: (i) homology model and validation for protein structure prediction, (ii) biochemical characterization of purified protein, and (iii) dose dependent enzyme activity	03
15	Substrate specificity and effect of substrate concentration	02
16	Effect of various factors on the enzyme activity	02
17	Thermal stability study	01
Total		41