

A REVIEW

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## Metagenomics approach in forest ecosystem

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**ABSTRACT :** Forest ecosystems come under some of the most complex microbial community on Earth. Forest soil is an assemblage of archaea, bacteria, fungi, and protists, who play a crucial role in biogeochemical cycles. The soil ecology, biogeochemistry, microbial community structure serves as an indicator of forest ecosystem status, energy flow pattern and soil productivity. But it is difficult to determine the microbial composition, diversity and variability of forest soil as about 99.5% of micro-organisms cannot be cultured and grown in laboratory condition. Metagenomics is a culture-independent genomic analysis of micro-organisms, directly obtained from the environment and can be used to tackle such problems which are assisted by rRNA universal markers, next-generation sequencing techniques, and bioinformatics tools. Attempts have been made by scientists to explore the forest microbiomes through metagenomics approach have been reviewed in this article. Metagenomics analysis has been carried out in various type of forest soils to access the microbial genetic resources and to know the phylogenetic diversity and abundance of a particular forest type, which helps in understanding their role in the forest ecosystem. As per the future line of work, it is needed to apply metagenomics in accumulating information across diverse forest habitat types which will help in better understanding the role of micro-organisms in the forest ecosystem, how they are helpful against different abiotic stresses, diseases, and other ecosystem functions.

**KEY WORDS :** Metagenomics, Next-generation sequencing, Bioinformatics tools, Forest ecosystem

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### INTRODUCTION

The forest ecosystem is the most important component of the biosphere, has contained a large diversity of flora and fauna, and is the home of many microorganisms, who play essential roles in biogeochemical cycles, water/air purification, soil conservation, and climate regulation (Lee, 2015). Forest

floor, composed of litters which include about 80% of the net above ground production (Kerekes *et al.*, 2013), provides fundamental ecosystem services to microorganisms such as fungi, bacteria, archaea, and small invertebrates to consume, decompose, recycling of nutrients (Schneider *et al.*, 2012). The microbial community is responsible for regulating soil productivity and resiliency against stress (Hartmann *et al.*, 2012).

The diverse abundance of micro-organisms in the forest ecosystem makes it too difficult to determine the microbial composition and function (Ross-Davis *et al.*,

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2014). The abundance of microbes in forest varies according to the type of forest, vegetation, climate, geographic location, and soil properties (Lee, 2015). The major part of forest soil ecosystem is occupied by prokaryotes, but only 0.5% of the total microbial population can be classified (Torsvik *et al.*, 2002). This is because most of the micro-organisms cannot be cultured in laboratories. So to discover the vast microbial population in the ecosystem, approaches are needed which should be beyond culture based traditional practices. It led to developing a new branch of microbiology, called metagenomics, which is a culture-independent method of microbial study (Hondelsman, 2004).

### Metagenomics:

Metagenomics/environmental genomics/community

genomics is a new approach towards microbial study strategy where environmental DNA/ DNA of all the organisms present in the particular environment has been isolated and utilised for phylogenetic or genetic studies without culturing in any medium (Handelsman, 2004). Metagenomics involves in two types of screening *i.e.* sequence-based screening and function-based screening. Sequence-based screening involves complete sequencing of clones or random sequencing of clones to indicate the taxonomic group that is a probable source of the DNA fragment, whereas function-based screening based on the expressivity of specific function through transcription and translation of gene or genes (Handelsman, 2004).

Use of universal phylogenetic markers (16 rRNA for bacteria, 18s rRNA and ITS for fungi and lower eukaryotes) helps scientist to construct phylogenetic trees

**Table 1 : Metagenomic approach to find out species abundance and functional diversity of different forest ecosystems**

Forest type	Microbial population studied	Approaches of sequencing	Key findings	References
Tropical rain forest	Bacteria	16s rRNA, Roche 454 Genome Sequencer	<i>Acidobacteria</i> , <i>Proteobacteria</i> , <i>Verrucomicrobia</i> , <i>Planctomycetes</i> and <i>Bacteroidetes</i> accounting for 90% of all Phyla	Miyashita <i>et al.</i> , 2013
Temperate forest	Bacteria	16s rRNA	<i>Acidobacteria</i> are dominant followed by <i>Proteobacteria</i>	Lim <i>et al.</i> , 2005
Temperate forest	Fungi	ITS 454 pyrosequencing	71% unclassified fungi found rest are dominated by <i>Ascomycota</i> and <i>Basidiomycota species</i>	Buee <i>et al.</i> , 2009
Tropical peat swamp forest	Bacteria	16s rRNA and Shotgun pyrosequencing	<i>Acidobacteria</i> and <i>Proteobacteria</i> are dominant	Kanokratana <i>et al.</i> , 2011
Montane forest	Bacteria	Plasmid end-sequencing	<i>Actinobacteria</i> , <i>Proteobacteria</i> and <i>Acidobacteria species</i> are dominated	Montana <i>et al.</i> , 2012
Subtropical low mountains hardwood and coniferous forest	Bacteria	16s rRNA	Themost abundant phyla <i>Acidobacteria</i> and <i>Proteobacteria</i>	Lin <i>et al.</i> , 2014
Neotropical rain forests	Fungi	454 pyrosequencing	The number of fungal taxa increased significantly with increasing mean annual precipitation, but not with plant richness	McGuire <i>et al.</i> , 2012
Western amazonian rainforests	Fungi	454 pyrosequencing	Fungal community structure is strongly linked with plants, both in terms of species composition and species richness	Peay <i>et al.</i> , 2013

**Table 2 : Capturing novel enzymes using metagenomic approach**

Enzymes	Source	Metagenomic approach	References
Antibacterial agent and indirubin and indigo blue compounds	Temperate forest	16s rRNA	Lim <i>et al.</i> , 2005
Lipolytic enzymes	Coniferous forest	General DNA manipulation and DNA sequencing	Lee <i>et al.</i> , 2004
Lipolytic enzymes	Peat-Swamp forest	Pyrosequencing	Bunternsook <i>et al.</i> , 2010
Lipases	Brazilian Atlantic forest	16S rRNA	Faoro <i>et al.</i> , 2011
Esterase	Colombian high andean forest	Functional-based screening	Jiménez <i>et al.</i> , 2012
Carboxylic ester hydrolases	Coniferous forest	Functional and sequential based screening	Biver and Vandenbol, 2013

of forest microbial ecosystem (Patwardhan *et al.*, 2014). Sequencing of DNA fragments isolated from environment is the next biggest challenge for researchers and is a time taking procedure, but with advancement of science and utilisation of next-generation sequencing (NGS) methods and tools (Roche 454 Genome Sequencer, Illumina HiSeq 2000, Applied Biosystems SOLID sequencer, and Ion Personal Genome Machine) make it possible to sequence large number of DNA fragments with less time and more accuracy, which minimize the time and cost consumption (Shokralla *et al.*, 2012). Again utilisation of bioinformatics tools such as IMG/MER, CAMERA, MG-RAST, and EBI metagenomics, provide a suitable condition for analysis, management, storage, and sharing of metagenome data (Oulas *et al.*, 2015).

### Forest types and metagenomics:

Estimation shows that the microbial diversity of forest soil is several thousand per gram of soil (Torsvik *et al.*, 2002), most of them found in the upper layer organic horizon (O horizon) (Baldrian *et al.*, 2012). The microbial community varies in their habitat in different forest types, also depends on tree species found in the forest, soil pH, soil texture, and soil type (Nacke *et al.*, 2011). The effect of forest types on microbial population is still unknown (Lee, 2015). Some of the abundance and functional diversity based studies of micro-organisms in forest ecosystem in different forest types are given (Table 1).

### Bioprospecting potential of forest metagenomics:

High microbial diversity in forest ecosystem often leads to the identification of novel enzymes, which can be derived by following the metagenomic approach of DNA isolation and functional based screening. Different studies on the discovery of novel enzymes from different forest ecosystem has been listed (Table 2). The discovery of novel enzymes from the forest is restricted by the fact that host specificity of microbes (Lee, 2015). A variety of host system has been developed to extend the potential of screening for *Proteobacteria* (Craig *et al.*, 2010), *Acidobacteria* (Sabree *et al.*, 2006). Though forest ecosystem consists of a great extent of microbial, the studies related to novel enzymes and biomolecules from forest metagenome is very less, so efforts must be given in this direction in future.

### Conclusion:

Forest ecosystem contains a great diversity of micro-organisms, play an essential role in forest nutrient cycle, but only 0.5% of total micro-organisms are known to us as they can be cultured in laboratory condition. Using metagenomics now it is possible to gain a depth knowledge on the abundance and functional diversity of micro-organisms in forest ecosystem. A very little knowledge has been gathered till now on this topic, as forest microbiology is neglected than any other environment. There has not been done any work on whole genome metagenome study, which can illustrate the bioprospecting potential of forest metagenomes. So much more future work is needed in this area.

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