Mycorrhizosphere- Unveiling the Hidden Life via Meta Genomics

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Abstract— In a wide range of terrestrial ecosystems, different symbiotic mycorrhizal associations between plants and fungi occur, almost ubiquitously. Historically, these have chiefly been considered within the slightly narrow perspective of their effects on the uptake of dissolved mineral nutrients by distinct plants. Mycorrhizal fungi connect their plant hosts to the heterogeneously dispersed nutrients required for their growth, permitting the flow of energy-rich compounds required for nutrient mobilization whilst concurrently providing conduits for the translocation of mobilized products back to their hosts. In addition to growing the nutrient absorptive surface area of their host plant root systems, the extra radical mycelium of mycorrhizal fungi delivers a direct pathway for translocation of photosynthetically derived carbon to microsites in the soil and a huge surface area for interaction with other micro-organisms. With phylogenetic surveys of soil ecosystems it has been known that the number of prokaryotic species in a single soil sample exceeds the known cultured prokaryotes. The soil environment is a plentiful yet under-characterized source of genetic diversity that has countless potential to enrich our understanding of soil microbial ecology and provide bioactive compounds and enzymes useful to human society. The complete functioning and regulation of these mycorrhizosphere processes is still poorly understood but current progress and the metagenomic studies are reviewed.

Keywords: arbuscular mycorrhiza, fungi, metagenomics, plant, rhizosphere

I. INTRODUCTION

Microorganisms residing in the soil have an important influence on fertility of soil and plant health. Soil microorganisms such as symbionts, saprotrophs and pathogens are substantial determinants of soil fertility and plant health. Better understanding of the interactions between these microorganisms with each other and with plants is a prerequisite for the efficient, sustainable management of soil fertility and crop production.

Fungi are heterotrophs, requires external sources of carbon for energy and cellular synthesis and they have implemented three different trophic strategies to obtain this carbon, occurring as necrotrophs, saprotrophs, and biotrophs. Fungi play a vital role in many microbiological and ecological processes, influencing soil fertility, cycling of minerals and organic matter, decomposition, as well as plant health and nutrition [16]. The zone of soil immediately adjacent to legume roots that supports high levels of bacterial activity is known as rhizosphere (according to [28] in [46]). The rhizosphere is characterized by amplified microbial activity encouraged by the leakage and exudation of organic substances from the root [22].

Mycorrhizas are symbiotic associations essential for one or both partners, between fungus and root of a living plant that is primarily responsible for nutrient transfer. The arbuscular fungi increases the absorptive surface area of their host plant root systems as well as the hyphae of these fungi provide an increased area for interactions with other microorganisms, and an important pathway for the translocation of energy-rich plant assimilates to the soil [33]. An important component of the microbial populations is formed by arbuscular mycorrhizal (AM) fungi which influence the plant growth and uptake of nutrients. Pale botanical and molecular sequence data propose that the first land plants formed associations with Glomalean fungi from the Glomeromycota about 460 million years ago [52]. This is assessed to be some 300–400 million years before the appearance of root nodule symbioses with nitrogen-fixing bacteria. Arbuscular mycorrhizal (AM) symbioses can be shaped with a very wide range of plant species, as many as 250000. Only 150–200 species of AM fungi have so far been distinguished on the basis of morphology, but DNA-based studies propose the true diversity of these symbionts may be very much higher [17, 57]. The symbiosis is categorized by highly branched fungal structures, arbuscules, which grow intracellular without penetrating the host plasma lemma [16]. Grasses like Trifolium alexandriunum also provides a rich niche for microbes and also helpful in phytoremediation [3].

The purpose of this review is to framework the current knowledge on microbial interactions in the mycorrhizosphere of AM plants and to study unculturable microbes that are useful for degradation of environmental pollutants. The review focuses on interactions between fungi and bacteria. In addition, it includes a brief discussion on metagenomic studies in mycorrhizosphere that how this knowledge of metagenomics or bioinformatics is currently used and how the understanding of microbial interactions could prove important to sustainable agriculture in the future.

II. MYCORRHIZOSPHERE

The mycorrhizosphere is the zone influenced by both the mycorrhizal fungus and the root, which also includes the specific term “hyphosphere”, that refers only to the zone surrounding individual fungal hyphae. Since mycorrhiza and fungal hyphae are more or less ubiquitous in natural soils, it could be contended that all soil could be included in the term “mycorrhizosphere”, Fig. 1 [33]. The term ‘mycorrhiza’ is derived from the Greek words ‘myces’ (fungus) and ‘rhiza’ (root). It was first used by Albert Bernhard Frank in 1885 [18]. He reported that ecto mycorrhizas are common in the root systems of woody plants in many types of soils. He suggested a symbiotic relationship between the plant host and fungus in which the fungus brings nutrients extracted from the soil to the plant and the plant nourishes the fungus by providing photosynthetically derived carbon substrates. The utilization of Nitrogen and Phosphorous from organic polymers, release of nutrients from mineral particles or rock surfaces via weathering, effects on carbon cycling, interactions with myco heterotrophic plants, mediation of plant responses to stress factors such as soil acidification, drought, toxic metals, and plant pathogens, as well as a range

of likely interactions with groups of other soil microorganisms [16].

The activity of plant roots has an influence on the biological activity as well as on the physicochemical conditions in the surrounding rhizosphere compartment and vice versa (Fig. 2).

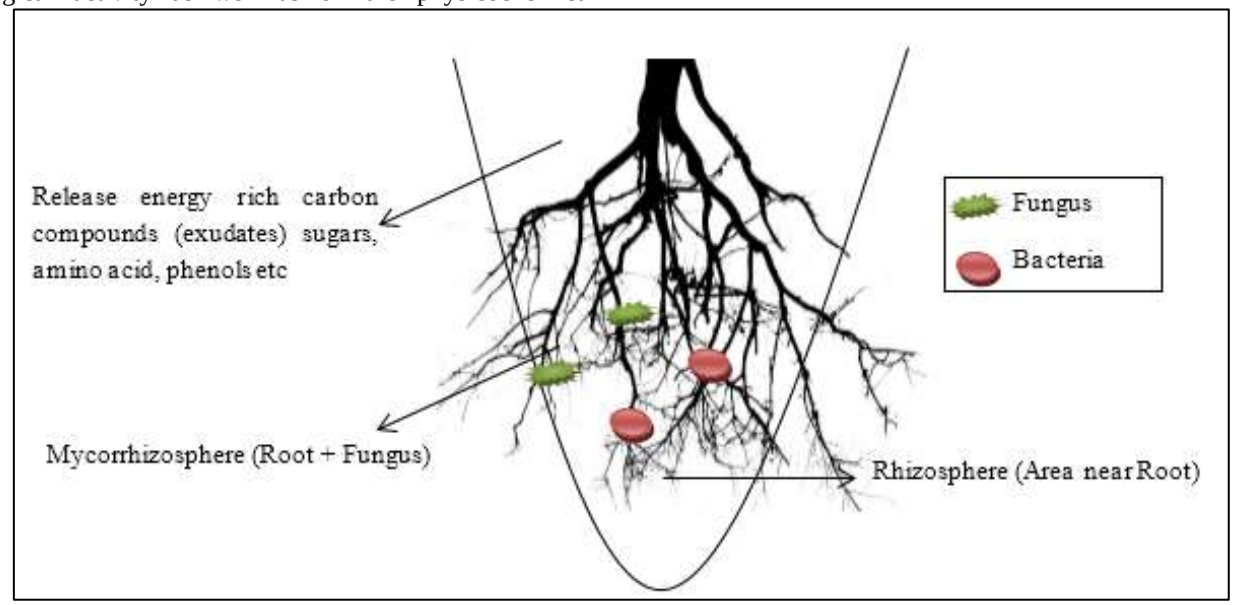

Fig. 1: Representation of mycorrhizosphere

III. Soil & Mycorrhizosphere

Soil is the major constituent of most of the terrestrial environments and is considered to be the most diverse ecosystem on Earth, in relation to its native microbial populations. It is estimated that one gram of soil contain millions of archaea, bacteria, viruses, and eukaryotic microorganisms [14, 69, 75], of which only a minor percentage has been cultivated in the laboratory [9, 30]. With phylogenetic surveys of soil ecosystems, it has been known that the number of prokaryotic species in a single soil sample exceeds the known cultured prokaryotes. The soil environment is a plentiful yet under-characterized source of genetic diversity that has countless potential to enrich our understanding of soil microbial ecology and provide bioactive compounds and enzymes useful to human society.

Mycorrhizas are roughly divided into three groups; ecto-,endo- and ectendo-mycorrhiza [63]. Endo-mycorrhiza, specially the arbuscular mycorrhiza (AM) are the utmost plentiful type of mycorrhiza. AM symbioses have been assessed to occur in over 80% of the flowering plant species on land [26].

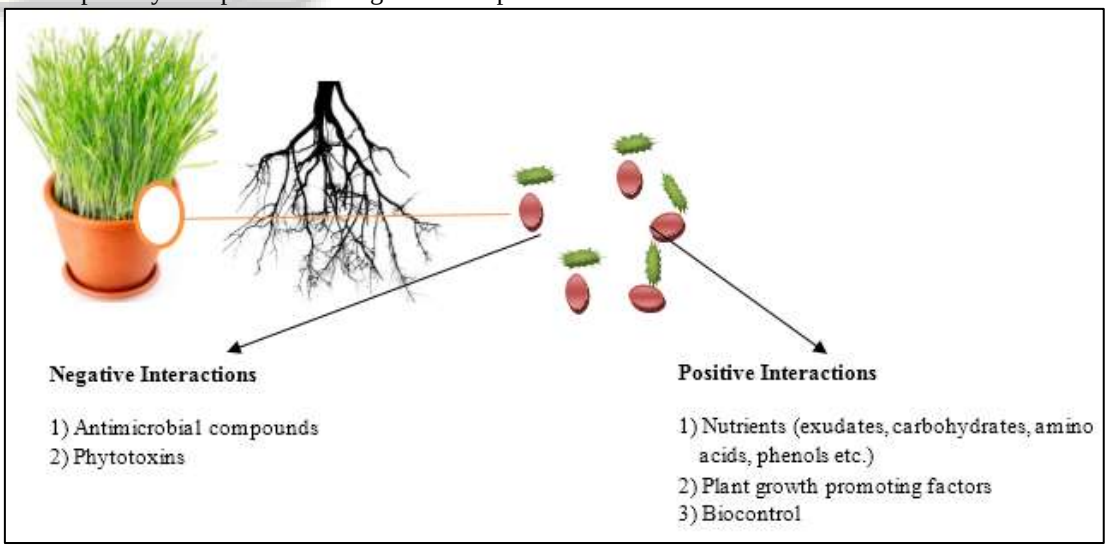

Fig. 2: Plant root interactions with bacteria and fungi

Mycorrhizal fungi have had a profound effect on the terrestrial colonization success of plants. There is fossil evidence for the occurrence of fungi in the roots of the earliest land colonizing plants [52] and it has been shown that AM fungi were present 460 million years ago, which predates the arrival of vascular plants [74].

The mycorrhizal fungi have significant impacts on the global carbon cycle. They have been shown to rise the rate of photosynthesis in the plants they colonize [29] thereby increasing the quantities of carbon the plant assimilates into its biomass and allocates into the soil. Not only is a significant

part of the plant derived carbon transported to the external mycelium, but carbon is also bound in the fungal biomass.

Mycorrhizal plants are often reliant on their fungal partners. There are studies where the symbiotic fungus has been shown to guard the plant partner from different environmental stresses, such as pathogens, drought and heavy metal pollution [26, 37, 63]. The benefits of the symbiotic fungus for the host plant and plant community are abundant.

IV. Cultured, uncultured bacteria & mycorrhizosphere

Rhizosphere communities are depends upon plant and soil factors, but slight is known about the comparative importance of these factors. The fact that no matter how many microbes we can see under a microscope or detect DNA in any given sample (soil, marine sediment, water, mucosa, etc), we can only successfully get around 0.1 – 1% to grow on Petri plates in the lab [12].

This severely limits what we can find out about how these organisms function out in the real world and has stalled many aspects of microbial ecology called as the great plate count anomaly. The new science- metagenomics can help to address some of the most complex environmental, agricultural, medical and economic challenges of today's world and has opens doors to a tremendous amount of scientific exploration. Further, it has been estimated that 4,000 different DNA units can exist in 1 g of soil. Also, hardly 1% of microorganisms of soil can be cultivated with classic laboratorial techniques and it is unknown if this percentage is representative of total microbial population [11].

Since the concepts of "mycorrhizosphere" and "rhizosphere" were coined it has been documented that microbial populations may differ in different fractions of soil and in the various zones of the mycorrhizosphere and the rhizosphere. Many earlier studies depend on dilution plate counts to enumerate and designate microbial populations. Such methods, however, only detect organisms that are cultivable. A large proportion of mycorrhizosphere bacteria remain unculturable, and it is therefore difficult to assess the microbial diversity in the mycorrhizosphere and the relative contribution of unculturable microorganisms to the interactions in the mycorrhizosphere. AM fungi themselves cannot be grown in pure culture or by standard techniques, but root organ cultures [2] that are normally used to culture and study AM fungi in vitro and can be used for examining the interactions of AM fungi with their biotic and abiotic environment [15, 64]. The study of rRNA genes has recently become a significant tool for studying the diversity of soil bacteria [41, 56] and mycorrhizal communities [10] in different ecosystems. The use of fatty acid patterns of lipopolysaccharides and phospholipids [76] and the utility of ergo sterols as bioindicators of fungi in soil [55] have also been valuable tools in the characterization of microbial communities. Genetic markers, e.g., gfp or genes coding for various forms of luciferase [32, 70], or viability stains have permitted direct counts of microorganisms using flow cytometry or microscopy, luminometry [5, 25].

A collective number of studies make use of polymerase chain reaction (PCR) based methods like terminal-restriction fragment length polymorphism (T-RFLP) [56] or denaturing gradient gel electrophoresis (DGGE) [41] for characterization of complex soil bacterial communities. The advantage of DGGE is that it recognizes very small differences in the nucleotide sequence, allowing a description of the community structure expressed as band patterns on a gel. The advantage of T-RFLP is that it tells the community structure without requiring culture or cloning. In common with DGGE, it gives a report of the species composition and estimates the relative abundance of taxa in the sample, based on the abundance of different restriction fragments detected by laser induced fluorescence on an automated gene sequencer.

Whilst the above methods enable the in situ study of microbial communities with an improved resolution, additional information is still often required about the functional capacities of identified taxa. Stable isotope profiling (SIP) provides a promising method for relating the fraction of the community that is functionally active in metabolizing a specific substrate containing one or several stable isotopes [49].

V. Bacterial Diversity- Genomics and Metagenomics

Like genomics, metagenomics is both a set of research techniques, which comprise of many similar approaches and methods. In Greek, meta means "transcendent." In this approach and methods, metagenomics overpowers the twin problems of the unculturability and genomic diversity of most microbes, the biggest roadblocks to progression in clinical and environmental microbiology. In the second sense, meta also recognizes the requirement to develop computational methods that make the most of understanding of the genetic composition and activities of communities so complex that they can only be sampled, not ever completely characterized.

Metagenomics, representing 'genomics on a vast scale' is a prevailing approach that permits an enormous valuation of the surplus of microorganisms present in the environment [4]. It is a new methodology that has revolutionized our understanding of microbial life existing on Earth. The application of metagenomic analysis has enhanced the quick rate of progression in the study of uncultured microbes that began with the advent of rRNA analysis.

VI. From Genomics to Metagenomics

Many projects of metagenomics have been using a version of the standard genomics analysis workflow: sequence as deeply as possible, assemble reads into consensus 'contigs,' and annotate these contigs [48, 54, 71]; however, this is a expensive strategy when using any of the current sequencing platforms. It is also significant that each analysis strategy equal the scientific goals of the study. For example, gathering into contigs, erasing singleton reads, and subsequently performing an analysis of the microbial communities will result in significant biases.

Due to the soil's chemical and physical heterogeneity, DNA isolated from soils is frequently co-isolated with organic compounds that can hinder downstream applications such as PCR and metagenomic library construction. Depending on the composition of the soil, these contaminants may comprise humic acids, polysaccharides,

polyphenols, and nucleases, which can also damage DNA [19, 67, 68, 77]. The eradication of these co-isolated contaminants is critical to positive DNA manipulation and extraction and purification methods should be selected to yield DNA suitable for the ultimate metagenomic application [34].

There are existing bioinformatics tools for prediction of gene, for example MEGAN (MEtaGenome ANalyzer), a program which compares a set of DNA reads (or contigs) against databases of known sequences that uses comparative tools such as BLAST (Basic Loccal Alignment Search Tool) algorithms. MEGAN can then be used to compute and interactively discover the taxonomical content of the dataset by using NCBI taxonomy that summarize and order the results [31]. Once a dataset of metagenomic sequences with significant GenBank hits has been assembled, these sequences can then be characterized by a subsystems approach using SEED to form predicted gene functions according to related biological processes [44]. SEED enables quick annotation of metagenomic sequences according to resemblance to previously known gene products. The predicted genes may also be allocated a phylogenetic classification using Treephyler for speedy taxonomic profiling of metagenomic sequences [58].

VII. Screening of Soil based on Sequencing

Sequence-based screening involves direct sequencing of metagenomic DNA, either with or without cloning prior to sequencing and then subjecting the sequences to bioinformatics analyses [36. 62]. There are two general approaches exist for environmental metagenomic DNA extraction: 1) DNA is extracted directly from the environmental sample; or 2) Microbial cells are recovered from the environmental sample prior to lysis and DNA purification [34] (i.e., "indirect extraction"). Direct extraction of metagenomic DNA has many benefits, including its reduced processing time and that it provides a higher DNA yield compared to other methods [43]. The analysis of metagenomic libraries encompasses two main strategies, sequence-based or function-based screening. The choice of screening method depends on various factors, including the type of library constructed, the genetic loci or functional activity of interest, and the time and resources available to characterize the library.

VIII. Next Generation Sequencing

Current metagenomics projects are simplified by the rapid development of so-called Next-Generation Sequencing (NGS) techniques [39] which offer lowered cost experimental tools without the cloning process inherent in conventional capillary-based methods. Next-generation sequencing has altered metagenomics. However, DNA sequencing is no longer the bottleneck; but, the bottleneck is computational analysis and also interpretation. Computational cost is the evident issue, as is tool limitations, considering maximum of the tools we routinely use have been made for clonal genomics or are being adapted to microbial communities. The recent trend in metagenomics analysis is toward decreasing computational costs through upgraded algorithms and through analysis strategies. Data sharing and interoperability among tools are serious, since computation for metagenomic datasets is very great.

With the result of the continuous and dynamic development of new generation sequencing (NGS) technologies along with the latest advances in methods to cope up with the metadata has resulted in the advancement of metagenomics. It has helped in the addition of our understanding of not only the diversity but also the functioning of the microbial communities. This qualitative and quantitative analysis of the environmental genomes have now abled scientists to unravel mysteries as well as to correlate the ones presented in entire microbial communities and the unexplained genes in sequenced genomes. Individual genotype assembly from a complex microbial community can actually reveal the amount of micro diversity and genome plasticity of that specific genotype or species.

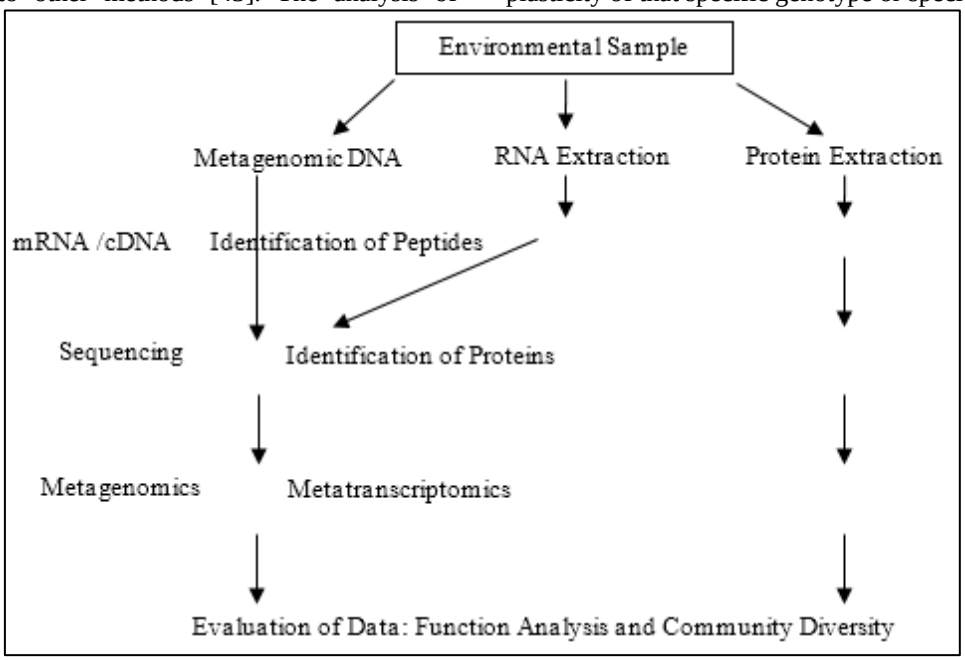

Fig. 3: General steps for evaluation of environmental sample

The development of NGS technologies, such as pyrosequencing of 16S rRNA genes, provided plentiful sampling deepness compared to traditional approaches, such as T-RFLP, DGGE, or 16S rRNA gene clone libraries. Though, the intrinsic error rate of pyrosequencing might result in the overestimation of rare phylotypes [60]. Direct sequencing of metagenomic DNA has been proposed to be the utmost accurate method for valuation of taxonomic composition [72]. The chief benefit of this method is the avoidance of bias presented by amplification of phylogenetic marker genes [60].

To date, metagenomic analysis have been used in numerous environments, and more than 210 different metagenomes have been sequenced from a huge variety of environments, such as global oceans, soil, the human gut, and feces [21]. The application of metagenomics was continually expanded in the recent year, such as (a) Mining of metagenomes from extreme environments, (b) Bio prospecting from metagenomic libraries, (c) Assessment of taxonomic and functional diversity of microbial communities [60] etc. Metagenomics provides abundant information on the metabolic and functional capacity of a microbial community [66]. However, as metagenomic DNA-based analyses cannot distinguish between expressed and non-expressed genes, it fails to mirror the actual metabolic activity [65].

Using metagenomic approach, Fan et al., 2012 isolated a novel thermo stable pyrethroids hydrolyzing enzyme which could be used in the detoxification of pyrethroids [13]. Following a similar metagenomic approach in cow rumen, a novel gene responsible for the degradation of 3,5,6-trichloro-2pyridinol, a persistent and toxic metabolite of the insecticide chlorpyrifos was isolated [40]. A bacterial isolate K4 identified and named as Stenotrophomonasmaltophilia MHF ENV20 was found surviving at high concentration of chlorpyrifos and utilizing it as sole source of carbon and the energy was determined as a potential degrader and further selected for bioremediation studies. The isolate Stenotrophomonas spp. will be potentially useful in bio treatment of waste water and bioremediation of soil contaminated with chlorpyrifos [35].

IX. Computational & Statistical Tool for Metagenomic Study

The initial attempts for assembling metagenomic sequences used conventional whole genome assembly (WGA) pipelines, which includes whole genome assemblers and gene finding programs initially designed for conventional whole genome shotgun sequence (WGS) projects with only some small parameter modifications. The progress of genome assembly algorithms has been increased recently by the development of NGS techniques. New genome assemblers for short reads, which includes Velvet (an Eulerian path assembler) [20, 27], ALLPATHS [7], Euler-SR [8] have been developed that are specifically targeted at small and ultra-short reads (for example, the 454 pyrosequencer and the Illumina/Solexa and SOLiD sequencers).

X. Computational challenges

The cost of computational techniques for metagenome sequence analysis is so high that only a subset of the traditional tool set from clonal genomics is normally applied. While finding short, non-coding RNAs is evidently interesting to many and might lead to abundant new insights into the biology of the biome studied [59], the computational cost is high. The cost for running BLASTX analysis for enormous datasets on Amazon's EC2 cloud is numerous times the cost of running the sequencing instrument [73], with sequencing cost reducing much faster than computing cost. Running analyses that are significantly much expensive than BLASTX, such as RFAM [23] or CRISPR [24] is not current practice, with the trend going in the direction of reducing the cost of computation.

Computational analysis has revealed an even better impact on metagenomic studies as compared to traditional genomic projects, due not only to the huge amount of metagenomic data, but also to the new complication introduced by metagenomic projects (e.g., assembly of multiple genomes simultaneously is further challenging than the assembly of single genomes).

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