

## Importance of biodiversity in rhizosphere microbes for crop response and methods to assess the biodiversity

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### ABSTRACT

Microbes play a vital role in influencing the quality and health of soil and plants. Several studies had led to understanding of diversity and structure in the plant rhizosphere: linking with Soil Microbial diversity to Modern Agriculture, amrita gupta et.al (2022); Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast, China Peiyu Luo et.al (2013). The microbial population has many applications and advantages which are determined only through Long term fertilizer studies. Some of the methods used for assessing the biodiversity of these rhizosphere microbes are: 16s rRNA sequencing, DDGE, TDGE, SEM, etc. Some advantages of rhizosphere microbes are improved crop yield, fighting climate change with minimal soil and environmental degradation, the protection of human health for current and future generations, macro and micro-nutrient cycling for optimum agricultural growth, higher crop yield, and also prevents land degradation. In this article we will study about importance of biodiversity of rhizosphere microbes and various methods to identify the microbial population.

**Keywords:** Long term fertilizer trials, PGPR, 16s ribosomal RNA, Rhizosphere microbes, etc.

In the early 1900's the studies based on rhizosphere microbes had started. The term rhizosphere was coined for the first time by L. Hiltner which was used to denote the area of intense microbiological activity in the soil which generally extends up to several millimeters from the root system of the plants. According to the scientists Balandreau and Knowles (1978) F.E. Clark [1949] and the rhizosphere is divided into 3 regions namely endorhizosphere, exorhizosphere and rhizoplane. Nowadays most of the agri-biotech techniques are focused on prioritizing crop commercialization and increasing the yield of crops for short term benefits without any foresight for sustainable development and natural balance instead of focusing on the ground level factor for improving the crop yield like the activity of soil micro-biome, which has led to facing many drawbacks like decreased nutrient composition of soil, soil erosion, soil acidification, recalcitrant xenobiotics, etc. The rhizosphere region has many positive impacts to the plants and some of them are: (a) the microorganisms catalyse the reactions in the rhizosphere and produce CO<sub>2</sub> and form organic acids that in turn solubilize the inorganic nutrients of plants. (b) Aerobic bacteria utilize O<sub>2</sub>, and produce CO<sub>2</sub>, therefore, lower O<sub>2</sub> and increase CO<sub>2</sub> tension that reduces root elongation, and nutrient and water uptake. (c) Plant growth regulators such as indole acetic acid, gibberellins, cytokinins, etc. are known to be produced by the rhizosphere micro-flora. (d) They influence phosphorus availability to plant through the process of mineralization and immobilization. However, when plant suffers from nutrient scarcity during summer in tropical areas the microorganism release the immobilized nutrients.

Therefore, they act as sink between soil and plant roots in nutrient poor systems, and many more.

The biodiversity of rhizosphere microbes in a particular soil can be studied and developed only by using long term trials. Organic and inorganic fertilizers are primarily used to increase the crop yield, and in short-term fertilizer (STF) experiments, they have no major effect on microbial community (Crecchio et al. 2001; Marschner et al. 2001). However, in long-term fertilizer (LTF) experiments, they can affect the function, community structure, and population of soil microorganisms (Marschner et al. 2003; Cinnadurai et al. 2013). The LTF trials help us understand the complexity of the soil microbes when the test field is treated with different types and composition of fertilizers which may be organic or inorganic. According to majority of the LTF studies conducted in different countries with different soil, climatic, nutrient, and environmental conditions its collectively accepted that the diversity of the rhizosphere microbes present in the soil sample is always rich if the particular test plot has been treated with organic fertilizers, and contrary to the above claim the test plot which has been treated with inorganic fertilizers has a less complex and simpler diversity of soil microbes.

The information generated from various aforementioned studies and other theories numerous scientists and institutions have come forth in supporting facts, stating that rhizosphere microbes indeed play an important role in controlling the growth and development of the plants (both commercially important and wild varieties). So, in order to enrich the diversity of soil microbes many countries have started to support and increase the use and production of organic fertilizers

which even though don't show any significant effect in the short term they are very helpful in the long term sustainable development of the crops and also help in increasing the yield of the crops.

Some of the applications of rhizosphere microbes are: They act as bio-control agents, inducers, stimulants, help in Induced systemic resistance (ISR), and can also be used on the commercial level as biofertilisers, bio-pesticide.

## MATERIALS AND METHODS

In general, biochemical (culture dependent) and molecular (culture independent) methods are used to assess the biodiversity of the rhizosphere microbes present in the soil.

Morphological identification such as cell shape, size, colour, flagella and gram staining are used to classify the microbes. Since only a very limited per cent of bacterial communities can be cultured, classification based on the morphological features are not enough to establish a detailed classification of bacterial communities in the soil.

There are also some other advanced molecular methods like shot gun sequencing, identification based on PCR, 16s rRNA, metagenomics, micro array based identification, etc. are used to assess the soil microbial biodiversity in recent decades.

Culture dependent method: here the microbial sample is inoculated into different culture mediums, in which each culture has different compositions to study the characteristics of the microbial population. It's a long and time consuming process. According to the scientific studies only 3% of the microbes found in soil can be cultured, so only few species or groups of microbes can be identified using these methods.

Culture independent method: in this method the rhizosphere microbial samples are not inoculated into any media but are characterised based on in silico analysis using bio-informatics studies and tools like SILVA, UNITE, NCBI, Kronatools, etc. The result is represented in the form of heat maps, venn diagrams, or krona charts which are designed on the basis of the bio-informatics studies.

But most frequently used method of identification for rhizosphere microbes is based on 16s ribosomal RNA of the microbes, through which 16s rRNA sequencing is done. The 16s ribosomal RNA is found in all the prokaryotes and has 9 hyper-variable(HV) regions(variable in every microbe) which plays a major role in taxonomic identification of the microbes along with other regions called as conserved regions(which are constant in all the microbes).

The most frequently used hyper-variable regions are V3 - V4 regions. But sometimes instead of choosing only the HV regions we sequence the whole sequence containing 1500bp or more, this method is called as shotgun sequencing. The primers used for this process differs based on the source organism from which 16s rRNA is taken from. This method is more prevalent than

others as it has highest accuracy compared with other methods.

16S metagenomics approaches, OTUs are cluster of similar sequence variants of the 16s rRNA marker gene sequence. Every cluster is intended to represent a taxonomic unit of a species or a genus depending on the sequence similarity threshold. Typically, OTU cluster are defined by a 97% identity threshold of the 16S gene sequences to distinguish bacteria at the genus level.

Species separation requires a higher threshold of 98% or 99% sequence identity, or even better the use of exact amplicon sequence variants (ASV) instead of OTU sequence clusters.

## CONCLUSION

In the early 1900's the studies based on rhizosphere microbes had started. The term rhizosphere was coined for the first time by L. Hiltner which was used to denote the area of intense microbiological activity in the soil which generally extends up to several millimeters from the root system of the plants. The biodiversity of rhizosphere microbes in a particular soil can be studied and developed only by using long term trials. The methods for assessing the biodiversity of rhizosphere microbes are biochemical (culture dependent) and molecular (culture independent) methods are used to assess the biodiversity of the rhizosphere microbes present in the soil and the culture independent methods are more favored as they can assess a wider range of soil microbes than the culture dependent method. Most commonly used method of identification these rhizosphere microbes is by 16s rRNA sequencing and followed by many others like DDGE, TDGE, SEM, etc. The 16s ribosomal RNA is found in all the prokaryotes and has 9 hyper-variable(HV) regions(variable in every microbe) which plays a major role in taxonomic identification of the microbes along with other regions called as conserved regions(which are constant in all the microbes). The most frequently used hyper-variable regions are V3 - V4 regions as they are best differentiating factor which help us in microbial identification.

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