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# 1 Rhizosphere Biology – an Overview

Chakravarthula Manoharachary, Krishna G. Mukerji

## 1.1 Introduction

Fungi, bacteria and actinomycetes are known to colonize diverse habitats and substrates and they are known to play substantial role in plant health and productivity besides producing diseases. The specialized ecological niches where the microbial association and their activity amply evidence are soil, rhizosphere, rhizoplane and phylloplane. These microbes may interact with the same plant simultaneously either independently, synergistically and/or antagonistically resulting sometimes in beneficial effect and at other times in harmful consequences. The microbes living in the complex region of rhizosphere influence the crop health and yield. The fungal biotechnologists have forgotten that soil, rhizosphere, rhizoplane and phyllosphere are the natural resources for microbial metabolites, products and other of biotechnological importance.

## 1.2 Soil Microbes

There is vast microbial flora inheriting the earth and they are found in all types of soils which are virgin or cultivated, sands, deserts, thermal soils or of volcanic origin, in bogs and moors, in snow covered soils, in sediments, semi aquatic ecosystems, on rocks and in rock crevices. The dominating groups of microorganisms are bacteria, actinomycetes, fungi, soil nematodes and protozoans. However, most of these organisms share a common character in being heterotrophic in their nutrition and thereby depend on other organic, dead or living organism and inorganic source of nutrition for their survival and multiplication. Most of the fungi, bacteria and actinomycete are microscopic and show vast variation quantitatively and qualitatively in different sites of collection and at different

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Chakravarthula Manoharachary: Department of Botany, Osmania University, Hyderabad 500007, A.P., India

Krishna G. Mukerji: Department of Botany, University of Delhi, Delhi 110007, India

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depths. Considerable variation occurs even between soil samples taken a few inches apart. Biotrophic, saprobic and symbiotic microbes are found in soil. The microbial population of soil is, therefore, dependent on composite micro-ecological environments. While most of the microorganisms live as saprophytes, others may be parasitic and symbiotic in relation to living organic substrates. Depending upon their enzymatic activity, they may be cellulolytic, keratinophilic, chitinophilic, pectinolytic, lignicolous or humicolous. The discovery of several parasitic fungi in causing root infection of several economically important crop plants paved way for intensive study of soil microorganisms in different niches-soil, rhizosphere or rhizoplane. Many of the parasitic fungi belong to genera *Pythium*, *Rhizoctonia* and *Fusarium*. Fusaria are known to cause root rots and wilts in young seedlings and some of them act as saprophytes on some dead plants tissues (Deacon 1996).

The discovery of several isolating techniques by several workers such as dilution plate techniques (Waksman 1911, 1944), soil plate method and modified soil plate methods (Warcup 1950), agar film method (Jones and Mollison 1948), immersion tube method (Chester 1948), immersion plate technique (Thornton 1952), baiting method (Harvey 1925), dilution frequency method (Allen 1957), direct microscopic examination (Conn 1981), uncoated glass slide (Rossi and Riccardo 1927; Cholodny 1930), root maceration (Stover and Waite 1953) and many other techniques for assaying of microbes resulted in their quantitative and qualitative estimations in different niches of the soil. Subsequently specific and nonspecific media were used selectively for isolation of bacteria, fungi and actinomycetes. Utilization of these isolating techniques and culture media contributed immensely in our present day understanding of soil, rhizosphere or rhizoplane microorganisms. The microorganisms in the soil are diverse and they differ quantitatively and qualitatively with the variations in the climatic, edaphic and biotic factors. The population dynamics of microflora of the soil depends on initial substrate and changes in substrate over period of time, nature and changes in other microflora and the genetic ability of a particular microorganisms to utilize or modify the components of the substrate and their interaction with biotic and abiotic components of the soil. Microorganisms in the soil are in constant state of flux and play an active role in recycling of organic wastes, nitrogen, phosphorus cycle and mineralization and other phenomenon and also produce several metabolites useful for humans. Bacteria from the soil were enumerated quantitatively by several workers. *Agrobacterium tumefaciens* was isolated by Schroth and Snyder (1961).

Soil fungi in relation to distinct fungal communities, physico-chemical factors, seasonal variations, biotic and other ecological parameters have been studied by several workers (Behera and Mukerji 1985a,b; Manohara-

chary 1977; Manoharachary and Rama Rao 1975; Mukerji 1966; Rama Rao 1970; Saksena 1967; Warcup 1967). Soil fungi in relation to microhabitat, interaction, physiology, biotic factors, role in decomposition of soil organic matter, antagonism, and role of dilution plate method were some of the facts reviewed extensively at International Symposium on ecology of soil fungi held at Liverpool (Parkinson and Waid 1960). Fungi isolated from different ecological niches including soils have been compiled in several publications by Barron (1968), Gilman (1957), Rai et al. (1961, 1963, 1964a,b,c, 1969), Rai and Mukerji (1962a,b).

The effect of genetically altered *Pseudomonas solanacearum* in soil and rhizosphere of tomato was studied by Hartel et al. (1990) and it was revealed in their studies that the genetically altered *Pseudomonas* slowed down the growth of unaltered *Pseudomonas* as a consequence of recombination. The biological control of soil-borne diseases of crops was achieved by treating soils with VAM fungi (Sharma and Adholeya 2000). It was observed that damping off caused by *Pythium* and *Rhizoctonia* on cucumber seedlings and bacterial wilt caused by *Pseudomonas solanacearum* was controlled by charcoal compost and VAM combination but control was unsatisfactory with VAM fungi alone. Microorganisms isolated from the soil and the rhizosphere of wheat plants produced growth promoting substances whose effect was similar to those of gibberellins (Voker and Birnstiel 1989).

Pigmented actinomycetes were isolated from Cerrado soils of Brazil using starch agar nutrient medium (Copelmas and Linhares 1993). The tests revealed that 90% of the pigmented actinomycetes were melanin producers. Melanin production was induced by supplementary inorganic or organic nitrogen source. These strains may have a role in soil organic matter formation. An actinomycetes strain – TB isolated from Korean soils – showed strong antimicrobial activity against Gram-positive bacteria and tumor cell growth inhibition (Jin-Ho et al. 1993).

Survey of actinomycetes from mud and water samples for a period of five years from 12 lakes in China was carried out by Jiang and Ku (1989) and they have found that the number and composition of actinomycetes was related to physical and chemical factors of the water body. Five new species *Micromonospora phaeovivida*, *Saccharomonospora yumnanensis*, *Microtetrastroma flavorosea*, *Actinomadura viridoflava* and *Micropholyspora* were reported.

## 1.3 Rhizosphere Soil

The rhizosphere may be defined as that portion of the soil which is adjacent to the root system of a plant and is influenced by the root exudates. The

area of this zone depends on the soil type and host plant under study and soil environment conditions. The roots exert influences on various type of microorganisms. The stimulatory effect on microorganisms is known as the "Rhizosphere effect" as indicated by the interaction of soil and rhizosphere microbes and their ratio. The chemical and physical nature of the root zone is quite different from the soil away from the root zone and the biology of this complex zone has been studied extensively. The term 'Rhizosphere' was proposed by Hiltner (1904). The phenomenon of accumulation of microorganisms around the root zone was reported by a number of earlier workers (Agnihotrudu 1955; Starkey 1958; Rouatt 1959; Parkinson 1957; Katznelson 1946; Sadasivan 1965; Jackson 1960; Mukerji and Ranga Rao 1968; Ranga Rao and Mukerji 1969, 1971a; Sorensen et al. 1997). Various compounds such as amino acids, vitamins, sugars, tannins etc. are exuded by the roots. These compounds have selective effect on microorganisms with in the root zone. Some root exudates are also known to affect certain microbial species adversely leading to their decrease in the root zone and, in return, microorganisms are known to exert profound influence on the plant itself by decomposition, affecting nutrient uptake, antagonistic effect on other microbes and parasitism.

Rhizosphere is a soil ecological region where soil is subjected to specific influence by plant root due to the exudates from root cells and sloughing of root tissue (Curl and Truelove 1986; Giddens and Todd 1984; Harley and Russell 1968). According to Pinton et al. (2001), rhizosphere represents a poorly defined zone of soil with a microbiological gradient in which maximum changes in the population of microflora in soil is evident adjacent to root and decline with distance away from it (Newman 1978; Bowen 1991; Mukerji 2002). The region of the external surface of plant root together with any closely adhering particles of soil or debris is differentiated from it and has been called rhizoplane. Root exudates stimulate microbial activity selectively in rhizosphere and rhizoplane regions (Bansal and Mukerji 1996). There is an intense competitive activity by the obligate saprobes, unspecialized root parasites and root inhabiting fungi depending on their behaviour towards exudates. In case of root diseases the pathogen has to react with the rhizosphere and rhizoplane fungi before entering the root tissues. These may show antagonism and check its advancement. The various factors and mechanism responsible for fungistasis, although already investigated, still need in-depth investigation. Plant microbe interaction is a regular and continuous feature of the biological world. The beneficial fallouts of such interactions have been extensively exploited for economic gain in recent years.

Once we develop a microbial technology for nif gene transfer and genes of mycorrhizal fungi and chitinase, genes offering resistance to plant pathogens, will definitely boost the crops production and pave the way

for sustainable agriculture, making man's efforts for freedom from hunger a realizable end. This technology will reduce artificial fertilizer application and pesticide use.

Many have emphasized the importance of rhizosphere in plant-microbe interaction (Pinton et al. 2001). It has been documented by several researchers that microorganisms are stimulated in the rhizosphere (Katznelson 1946, 1965). Significant differences in the rhizosphere effect have also been reported. The metabolic state of the plant and the nature of soil appear to influence the extent of the rhizosphere effect (Rovira 1991). There is a need to learn more about the beneficial rhizosphere microorganisms. Ultimately the work on rhizosphere microbes has to aim at augmenting the biomass production (Yang-Ching et al. 2000).

Rhizosphere studies are fascinating and interesting leading to many beneficial consequences though some microbes have harmful effects. Some microbial metabolites like antibiotics and toxins, are crucial factors in determining plant-microbe relations. Microbial enzymes also play an effective role. The interesting relationship between plant root and microbes has attracted the attention of molecular biologists, microbiologists and biochemists throughout the world. Interestingly different types of microbes like fungi, bacteria, nematodes and viruses may interact with the same plant simultaneously either independently, synergistically or antagonistically.

Factors such as soil type, soil moisture, pH, temperature, plant age, relative humidity and several other factors are known to influence the rhizosphere effect. The rhizosphere effect is expressed in terms of R/S ratio (Katznelson 1946; Timonin 1966). The term 'rhizoplane' was proposed by Clark (1949) to refer to the immediate surface of plant roots together with any closely adhering particles of soil or debris. Using different isolation techniques microorganisms were isolated and identified by a number of workers.

In recent studies on rhizosphere, the emphasis was laid on interactions of microflora in the root zone due to effect of systemic or aerial sprays of herbicides or insecticides or fungicides on rhizosphere microflora (Ranga Rao et al. 1972). Data on microflora in Relation to various economically important crop plants or medicinal plants, studies on root diseases (Ranga Rao and Mukerji 1971b), soil environment and various factors of biotic, edaphic, environmental variations on the rhizosphere microflora and Dynamics Of Microorganisms In The Rhizosphere are meagre.

Mineralogical studies of rhizosphere of forest plants from six different sites in north and south of United States (Lynch and Whipps 1991) revealed that mineral grains within these zones are affected mechanically, chemically and mineralogically by invading root microorganisms than compare with non-rhizosphere forest soils. The mineral grains abutting root surfaces

were significantly more fractured. This process of weathering in the root zone micro-environment should have a positive effect on soil complex formation that is biologically active.

Using soil biology methods on rhizosphere soils of six different plant species grown in four different soils and combinations of species were studied (Lynch 1982) to get information on organic metabolites and their origin in the rhizosphere. The amounts of sugar, amino acids and enzyme activity of some metabolites suggested a closer interrelationship with the involved organisms. Sometimes the nature of the soil was the dominating factor while it was host plant species which played a major role.

Recently plant growth promoting rhizobacteria (PGPR) were isolated from a number of economically important plants such as barley, bean, cotton, maize, groundnut, rice, various vegetables, wheat and wood species. In addition to increasing crop yield the strains of PGPR can also affect pathogenic fungi in reducing population densities.

With a higher nitrogen input a higher amount of  $^{14}\text{C}$  was released from roots as photosynthates. This indicates that root exudates were significantly used by soil microbes in the rhizosphere. Using different nitrogen regimes, the rhizosphere bacteria of wheat was investigated (Keister and Cregan 1991). The bacterial population in relation to different nitrogen concentrations in the soil application resulted in higher amounts of bacterial populations in the rhizosphere but fluorescent pseudomonas population were low at higher nitrogen rates. The number of species of soil microorganisms, such as bacteria and actinomycetes were significantly correlated with cumulative temperature and rainfall in different agroclimatic zones. Similarly, higher correlations were obtained between rhizosphere microorganisms and pH, K, Ca and Mg. *Acremonium*, *Alternaria*, *Cladosporium*, *Cephalosporium* and *Fusarium* were the dominant fungal species.

Molecular cross talk seems to be the prerequisite mechanism for most root microbial infections. Just as the rhizodeposition can affect the composition of rhizosphere microflora, microbial metabolites can also affect the rhizosphere deposition. Rhizobacteria promote plant growth due to the production of plant growth regulators like auxin derivatives. The most studied molecular cross talk has been between rhizobia and the leguminous and non-leguminous host plant. The root exudates or chemical composition of rhizosphere solution can affect plant growth. It is very much the case that uptake of nutrients may be considerably influenced by the ionic concentration of the rhizosphere solution. However, we do not have the complete picture that takes into account the relative weight of each factor regarding molecular analysis of the interaction between plants, microbes and soil components.