

Role of Microsymbionts in Plant Microbe Symbiosis

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Abstract

Symbiosis refers to the long-term relationship of two unlike species. Microbes symbiotically associate with plant for several reasons, most importantly, to utilize carbon compounds from the root exudates as nutrients. Most of the microsymbionts acknowledge plant provided nutrients via benefitting plant by converting atmospheric nitrogen to the plant usable form of nitrogen, solubilizing unavailable form of phosphate and zinc into available form, providing phytohormones for better plant health, and protecting plant from the pathogens and abiotic stresses. Considering the beneficial properties of plant associating microsymbionts, it could be used as biofertilizers at commercial scale. Biofertilizer is an ecofriendly solution to minimize the hazards of chemical fertilizers. Microsymbionts exert their positive impact on plant once they appropriately colonize rhizosphere or invade plant root or nodules. This review indicates that how microsymbionts colonize plant and contribute to the plant health and crop production.

Keywords: Nodulation; Nitrogen fixation; Phytohormones production; Phosphate solubilization; Biological control

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Introduction

Plant microbe symbiosis has significant importance considering its crucial role in nutrient cycling. Microsymbionts of this relationship, microbes, display several important plant growth promoting features. Specifically, most of the rhizosphere-associated microbes have the potential for plant growth stimulation and managing soil health by establishing a symbiotic relationship with the compatible plant. Most extensively studied group of plant beneficial microsymbionts is known as plant growth promoting rhizobacteria (PGPR), which generally colonize the plant root surface and soil adhered to root. Some opportunistic PGPR can enter the root interior, pass through the root cortex and proliferate as endophytes in nodules, tubers, stem, leaves and other organs of plant [1-3]. These bacteria either on the root surface or the plant interior, selectively adapt to the environment and establish a symbiotic relationship with plant [4]. Plant secrete a substantial amount of the carbon fixed, 5-21%, mainly as root exudates, which is the chief source of attraction and nutrition for microbe and initiate a symbiotic relationship with microsymbionts. Microsymbionts in response promote plant growth by making the nutrients available, phytohormones production and controlling plant diseases [5].

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Nodulating microsymbionts

Root-nodulating and nitrogen fixing bacteria are collectively termed as 'rhizobia' and traditionally belong to one of the six genera; *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium* and *Rhizobium* [6]. Molecular data have shown that rhizobial bacteria actually constitute a polyphyletic assemblage of *alpha*, *beta* and *gamma* *proteobacteria*, which collectively form the order *Rhizobiales* [7-9]. The rhizobia-legume symbiosis exhibits great host specificity, but so far complete information of host specificity is not available. Nodulation factor produced by rhizobia are the key components for the host specificity. A major breakthrough in rhizobia-

legume symbioses research was the identification of Nod factor structure from *Sinorhizobium meliloti* [10]. Currently, Nod factor populations and structure produced by rhizobial strains varies depending upon the type of rhizobia and its geographical location. Nod factors are lipo-chitoooligosaccharides, which are considered as molecular keys for the entry of microsymbionts into the legume host. Nod factors generally consist of a backbone of three to five rings of β -1,4-linked N-acetylglucosamine (G1cNAc), which is decorated at nonreducing-terminal with N-methyl, O-acetyl, N-acylated or O-carbamoyl groups and reducing-terminal carry acetyl, L-fucosyl, sulfate ester, 4-O-Ac-fucosyl or 2-O-Me-fucosyl (Figure 1) [11].

Interestingly, some Nod factors have exceptional length and type of G1cNAc backbone and diversity in its terminal's substitutions. Nod factors isolated from *M. loti* NZP2213 consist of a dimeric chito-oligosaccharidic backbone [12], whereas a pentameric Nod factor whose middle G1cNAc residue is replaced by a glucosyl group is explored from *Sinorhizobium fredii* [13]. *Rhizobium* sp. GRH2 synthesizes Nod factors consists of six G1cNAc residues [14]. Variable backbone length and substitutions at the reducing end also determine Nod factor host specificity [11]. Perception of Nod factor on plant root surface elicits a series of responses including root hair deformation, membrane potential depolarization, changes in ion fluxes, intracellular and extracellular alkalization, induction of nodulin gene (*ENOD*) expression and nodule primordium development, which finally results in the mature nitrogen-fixing nodules formation [15].

Associative microsymbionts

Associative microsymbionts mostly live in the phyllosphere, rhizosphere and rhizoplane or sometimes reside inside the plant tissue and organ. Rhizosphere and rhizoplane associated bacteria are termed as rhizobacteria, whereas those strictly found inside the plant are known as endophytic bacteria. The term rhizoplane refers to the root surface, whereas, the rhizosphere is the immediate thin zone of soil surrounding the root. Legume root nodules also accommodate various non-nodulating plant growth promoting endophytic bacteria, which influence the survival of rhizobia and impact the nodulation and grain yield of crop [16]. The influence associative bacteria may be passive, but most often they promote plant growth. Phyllosphere refers to the aerial part of the plant that also carries microorganisms. Bacteria are the most extensively studied microorganisms in this part of plant. Rhizosphere and rhizoplane associating bacteria generally

reside in the soil and move towards the plant as it produces root exudates. Microsymbionts attach themselves with to the root surface via cell surface polysaccharides or cell structures including fimbriae and flagella. Bacteria, cyanobacteria, fungi and algae reside in and around the root zone but bacteria is the most abundant [17]. The associative microsymbionts establish a relationship with plant for nutrient and shelter and in return promote plant growth mainly by fixing nitrogen, indole acetic acid production, phosphate solubilization, biocontrol of plant diseases and by many other mechanisms, which are being explored.

Characteristics of microsymbionts

Biological nitrogen fixation: Nitrogen is one of the most important plant nutrient applied exogenously to fulfill the world's food demand. Extensive application of chemical fertilizers has resulted serious hazards to the environment and human health. Nitrogen fixed by bacteria is environment friendly, replenishes nitrogen content of the biosphere and important for sustainable agriculture. Biological nitrogen fixation "the conversion of atmospheric nitrogen into ammonia" by the plant microsymbionts is a process of tremendous importance for food security and environment. These nitrogen-fixing bacteria are categorized as diazotrophs. Some bacteria have the ability to fix nitrogen as free N fixer, while other associate plant and develop nodules on the plant roots. Inside the nodules, oxygen free environment is created with the help an indigenous protein leghaemoglobin. Anaerobic bacteria, rhizobia, fix atmospheric nitrogen in this compartment. Nitrogen fixed by biological methods is eco-friendly and does not leach down or evaporates as nitrous oxide to pollute environment. Nitrogen fixation system and machinery does not exist in eukaryotes. The ability to fix nitrogen is distributed amongst a few microorganisms. Nitrogen fixation ability is found in most Eubacterial phylogenetic groups, including Actinomycetes, Cyanobacteria, Proteobacteria, green sulphur bacteria and Firmibacteria. Only a very few *Archaea* have the ability to fix nitrogen [18].

The enzyme responsible for the conversion of atmospheric dinitrogen to ammonia is known as Nitrogenase. It is a complex metallo-enzyme consisting of two components, smaller dimeric component functions as an ATP-dependent electron donor called iron (Fe) protein, and larger hetero-tetrameric component contains catalytic site of the enzyme called molybdenum-iron (MoFe) protein. Both of the nitrogenase component proteins

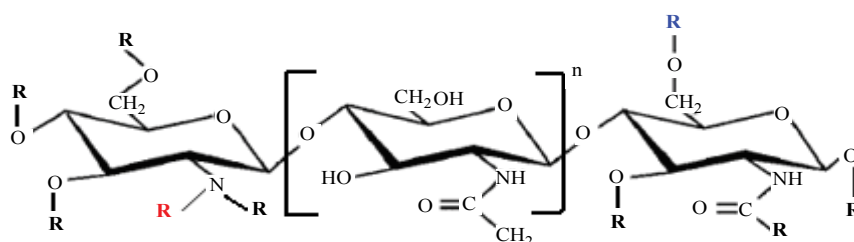
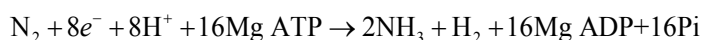


Figure 1 General structure of Nod factor.

are extremely oxygen sensitive [19]. The overall stoichiometry of dinitrogen reduction in optimal conditions is as under;



Indole acetic acid production: Indole-3-acetic acid (IAA) is one of the major hormones belongs to the auxins family. It is an important hormone that plays a significant role in many aspects of bacterial life cycle including virulence induction, cell cycle regulation and acid resistance. It also controls many important physiological processes including cell enlargement, cell division and tissue differentiation in responses to light and gravity. By modifying certain conditions like, increase in osmotic content of cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis and protein synthesis, IAA can stimulate cell elongation. IAA can also inhibit abscission of leaves and induce flowering as well as fruiting [20]. IAA is important for young seedlings as it enhances their ability to anchor themselves to the soil and to obtain maximum water and nutrients from their surrounding environment, so they may able to survive. It was later found that various microorganisms including bacteria, fungi, and algae are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment [21]. IAA can be produced by many rhizospheric bacteria that inhabitate plants. Due to the rich supplies of substrates exuded from the roots of plants, these rhizospheric bacteria synthesize and release IAA as secondary metabolites that directly promote plant growth. Tryptophan is generally considered as a precursor of IAA. The precursor of auxin as present in root exudate is usually synthesized from the exudate amino acid called tryptophan. IAA is the common product of L-tryptophan metabolism produced by several microorganisms that includes PGPR. The tryptophan concentration in exudate differs strongly among plants. Production of IAA by microbial isolates varies greatly among different species and strains of the same species and is influenced by culture condition, growth stage, and availability of the substrates [22]. The IAA produced by bacteria colonizing the rhizosphere of plants is proposed to act in conjunction with endogenous IAA in plant to stimulate cell proliferation, elongation, and enhancement of host's uptake of minerals and nutrients from the soil [23]. In addition, IAA serves as a regulating agent for microbial cell differentiation; for example, it stimulates spore germination and mycelial elongation in a *streptomyces*, and induces adhesion and filamentation in *Saccharomyces cerevisiae* [24,25].

Phosphate solubilization: Phosphorus (P) is major essential macronutrients for plant growth activity. One of the major constituent to mobilize energy in the plant and participate in functional integrity of nucleic acids. P is instituted in the soil in two basic formats, organic and the inorganic. The larger fraction of P prevailed in the soil in insoluble reservoirs include hydroxyapatite, and apatite. The deranged soluble P in soil matrix blemish the plant life activity. To recuperate the plant life activity towards betterment soil need to be provided with avenue of P most readily endorsed by plant. For the flourishing trade of soluble dish of phosphate to the soil is made available by chemical fertilizers, therefore P is applied to soil in the form

of phosphatic fertilizers. During the aisle of chemical fertilizers in the soil and over time majority of the fertilizers embedded in the insoluble pool of P in the soil matrix. These insoluble preserve include tricalcium phosphate, aluminum phosphate, and dicalcium phosphate.

Revitalizing plant with a substantial fraction of the inorganic Phosphorus unfortunately is claimed by immobilized pool of P in the soil matrix [26]. Soil microbes house a noble trait that can recuperate the immobilized P into Phosphate. The soil microorganism maneuver through exhaustive solubilization and mineralization, read the soil for inorganic P and ultimately regenerate in soluble form [27]. Cofactor pyrrolquinoline quinone (PQQ) is found to be involved in P solubilization. The current perspective is to rescue soluble P loss, and score a quality production. This refreshing attribute of phosphate solubilization has increased the participation of agronomists in deploying the phosphate solubilizing bacteria as inoculums [28]. The avarice of bacteria to solubilize phosphate has positive influence on Plant life cycle. This compliance of bacteria in the agro-ecosystem has poured a key advantage over the application of chemical fertilizers due to effectiveness, and low cost [29].

Biological control: Annual crop losses due to plant diseases are responsible for a total value of more than 200 billion dollars. Resistant plants and chemicals are often used to control plant disease. Resistance does not exist against all diseases and the breeding of resistant plants takes many years. Moreover, acceptance of genetically engineered resistance is still a sensitive issue in the European Union as so many risk factors are involved. Consumers and supermarket chains are negatively perceived the use of agrochemicals. Unprecedented use of agrochemicals results various negative effects including pathogen resistance and elimination of non-target organism. Most of the agrochemicals applied do not reach the plant. Moreover, consumer desire for pesticide free food and the increasing price of pesticides, especially in poor areas of the world, has led to a search for alternative methods to protect crops. Additionally, Chemical solutions are hardly few, inefficient or nonexistent for many particular diseases. It is ever more prohibited by governmental policies [30].

Biological control, reduction and elimination of pathogen using living organism, is an attractive and environment-friendly solution to combat plant disease and reduce the use of chemical pesticides. Microorganisms are natural competitor of the pathogen. Microbes produce secondary metabolites, locally, onto or near the plant surface to eliminate or inhibit the growth of pathogen [31]. These secondary metabolites are biodegradable and do not cause environmental pollution, compared to the agrochemicals that are designed to resist degradation by microbes. Rhizobacteria are usually studied for antagonism against pathogenic bacteria or fungi. Some rhizobacteria perform antagonistic activity against insects and weeds also [32-34].

Enzyme 1-aminocyclopropane-1- carboxylate (ACC) deaminase present in PGPR functions to promote plant growth by reducing the quantity of ethylene in plant. ACC deaminase producing bacteria take up the ethylene precursor ACC and transform it into

2-oxobutanoate and NH₃. There are many forms of stress that are relieved by ACC deaminase producers, like resistance to stress from polyaromatic hydrocarbons, from heavy metals such as Ca²⁺ and Ni²⁺, from salt and draught and effects of phytopathogenic bacteria [34,35].

Conclusion and Future Aspects

Plant symbiotic microbes could be isolated from the soil, rhizosphere, plant interior, nodules and could be used as biofertilizers. Microsymbionts improves plant health and

crop production as well as enhances soil fertility. One of the important and emerging characteristics of microsymbiont is their antagonistic nature that prevents the plant from various diseases. Researchers are working on biofertilizers since many decades, generated fruitful results and commercially viable products. Microbial inoculants are stable for long term in suitable field environment and contribute major role in sustainable agriculture. There is continuous need to explore new and novel microsymbionts as environmental changes occur over time and existing microbiota may lose it potential.

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