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REVIEW PAPER

The Interaction between Endophytic Actinomycetes and Rhizobium in Leguminous Plants

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Abstract

Biological N_2 fixation represents the major source of N input in many agricultural soils including those in arid regions where little artificial fertilizer is applied. The major N_2-fixing systems in agriculture are the symbiotic systems, where bacteria such as rhizobia interact with legumes to fix atmospheric nitrogen which plays a significant role in improving the fertility and productivity of low-N soils. The symbiotic association of legume-rhizobium is initiated by the colonization of the rhizosphere by the rhizobia and subsequent attachment to the root hairs of the host plant. Furthermore, the host will produce flavonoids, such as luteolin in alfalfa and diazedin in soybean, which interact with nod protein in the rhizobia. Moreover, this process then elicits the expression of a cluster of nodulation genes such as *nod, nol,* and *noe* in the rhizobia. The interaction is potentially of great importance to the health and growth in nature of this nodulating legume.

The interaction between endophytic Actinomycetes and rhizobia in leguminous plants is one way to improve the capability of leguminous plants to fix atmospheric nitrogen in plant roots and contribute to the plants nutrition. From other studies, we know that certain types of Actinomycetes, for example Streptomyces, interact with peas to form healthy roots as an effective site to form nodules and improve biological nitrogen fixation. Knowledge about this activity against fungal pathogens might lead to finding biocontrol agents for use in sustainable agricultural practices.

Root-colonizing soil borne Actinomycetes might influence root nodulation in leguminous plants by increasing root nodulation frequency, possibly at the sites of infection by *Rhizobium* spp. Actinomycetes also colonize and sporulate within the surface cell layers of the nodules. This colonization leads to an increase in the average size of the nodules that form and improves the vigor of the bacteroids which generate the red color within the nodules by enhancing nodular assimilation of iron and possibly other soil nutrients.

Keywords: symbiotic, biological, nitrogen, molecular interaction

Introduction

A symbiotic, associative, or symbiotic biological nitrogen fixation (BNF) is a free and renewable process (Jensen and Nielsen, 2003) which should constitute an integral part of sustainable agro-ecosystems. Yet there has been a rapid increase in use of fertilizer N and a parallel decline in the cultivation of leguminous plants and BNF.

BNF in various agro ecosystems has been extensively reviewed (Boddey et al., 1998; Giller and Wilson, 1991; Ladha et al., 1996; Ledgard, 2001) and since atmospheric N_2 is an almost inexhaustible resource, BNF is a sustainable source of N in agricultural cropping systems

As BNF is largely restricted to occurring in legumes, replacing fertilizer N in agricultural systems with symbiotically fixed N_2. BNF may require a legume crop to be grown as a green manure crop before, for example, maize, intercropped with maize, or grown alone. N_2 fixed by heterotrophic diazotrophs in sugarcane (Boddey et al., 1995) or *Anabaena azollae* applied in flooded rice may complement soil and fertilizer N as sources of nutrient for these crops.

Biological Nitrogen Fixation and Its Use in Agriculture

Nitrogen Cycles

The Nitrogen (N) and carbon (C) cycles are regarded as the driving forces in acidification of farming soils (Bolan et al., 1991; Helyar, 1976). Transformations of N, fixation of N by legumes, leaching of nitrate (NO_3), and the effects of ionic forms of N taken up by plants are particularly important processes in the gain and loss of organic matter.

Nitrogenize Reaction

The Society for General Microbiology called nitrogenize an enzyme which catalysis the conversion of nitrogen gas to ammonia in nitrogen-fixing organisms as one of the critical enzymes in nature. In legumes, nitrogenize only occurs within the bacteroids and the reaction requires hydrogen as well as energy from ATP. This nitrogenize complex is sensitive to oxygen, becoming inactivated when exposed to it. However, this is not a problem with free living anaerobic nitrogen-fixing bacteria such as *Clostridium* because they have a variety of different mechanisms to protect nitrogenize complex, including high rates of metabolism and physical barriers (Sprent and Sprent, 1990). *Azotobacter,* for example, overcomes this problem by having the highest rate of respiration of any organism, thus maintaining a low level of oxygen in its cells.

The nitrogenize reaction is supplied with energy in the form of ATP and reducing power from electron (e-) carriers, usually ferredoxin:

Rhizobium and *Bradyrhizobium*

Rhizobia (species of *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium*) form intimate symbiotic relationships with legumes by responding chemically to flavonoid molecules released as signals by the legume host. These plant compounds induce 'expression of nodulation' (*nod*) genes in the rhizobia, which in turn produce lipo-chito-oligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Dakora, 1995; Lhuissier et al., 2001).

Bradyrhizobium japonica was first isolated from a soybean nodule in Florida in 1957. *Rhizobium* sp. NGR234 has a host range of more than 112 genera of legumes (Viprey *et al.,* 2000). Rhizobia can also be found in the roots, or rhizosphere, where they cause the formation of nodules.

The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legumes, in terms of biological nitrogen fixation, has been a main focus of interest in the past. Rhizobia are known to cause nodulation and increase nodule weight in legumes along with an increase in growth and development of the host plant (Kraus et al., 1987), and this nodulation requires temporal and spatial regulation of genes and gene networks (Hayashi et al., 2012). In addition, they protect the roots from pathogen attack due to production of diverse microbial metabolites like siderophore (Kloepper et al.,1980), rhizobitoxin, plant growth enhancement through IAA production, and uptake of phosphorus and other minerals (Gholami et al., 2009).

Actinorhizal Plants

Actinorhizal root nodules result from the interaction between nitrogen-fixing Actinomycetes called *Frankia* and the roots of dicotyledonous plants belonging to 8 plant families and 25 genera (Benson and Silvester, 1993). These plants offer striking differences with the *Rhizobium*-legume symbiosis (Franche et al., 1998; Wall, 2000). While *Frankia* is filamentous, branching, gram-positive Actinomycetes, Rhizobia are gram-negative, unicellular bacteria. *Frankia* can interact with a diverse group of dicotyledonous plants (Diagne et al., 2013) whereas Rhizobia only form symbiotic relation with plants from the legume family and with one non-legume.

The functional relationship between *Frankie* and plants is far from simple. The microbe and the plant may show complete compatibility as far as establishment of infection is concerned, but the resulting association may not provide optimal benefit to either partner.

Symbiotic Nitrogen

The association between the legume host plant including peas, lentils, and alfalfa and the nodule bacteria is mutually beneficial (symbiotic), due to fixation of atmospheric nitrogen and its availability to the plant. The progress in using the rhizosphere bacteria, including their mechanism of action related to plant growth-promoting traits has been reported by Bhattacharyya and Jha (2012) and Shahzad et al. (2010).

The legume-rhizoid symbiosis is initiated by the exchange of signal molecules between the plant and the microbe (Trevaski et al., 2003), and then flavonoid compounds released by the plant attract rhizobia and trigger the production and release of nodulation (nod) factors by the bacteria (Peter et al.,

2002). Nod factors are specific lipo-chito-oligosaccharides (Lerouge et al., 1990) that affect the host plant in a number of ways. The first visible effect of Nod factors on the plant root is the curling of root hairs. Nod factors also trigger cell division in cortical cells, which leads to the formation of the nodule meristem (Cohn et al., 1998; Denarie et al., 1996).

The symbiotic legume-rhizobium relationship is initiated by the colonization of the rhizosphere by the rhizobium and subsequent attachment to the root hair of the host plant. Furthermore, the host will produce flavonoids, such as luteolin in alfalfa and diazedin in soybean which are products that will interact with Nod in Rhizobia. Moreover, this process then elicits the expression of a cluster of nodulation genes such as *nod, nol* and *noe* in the Rhizobium (Denarie et al., 1996)

Molecular Interaction of Leguminous Plants and Rhizobia

Rhizobia elicit the formation of new organs, called nodules, on their leguminous host plants, in which they fix nitrogen. The early steps in the symbiotic reactions between the rhizobia and leguminous plants are mediated by the bacterial secretion of substituted lipochito oligomers, the *nod* factors (Prome et al., 1998). This lipo-chito-oligosaccharide signal is produced by the bacteria in response to the phenolic acids produced by the host plant. Flavonoids, the inducers of *nod* genes in rhizobia are chosen in evolution because they are unique selected markers for the hormonal balance of the root (Bladergroen and Spaink, 1998). Flavonoids are produced and sent out by legume plant roots and attract both mutually beneficial and pathogenic to the roots (Phillipot et al., 2013), but only some of their constituents act as signals that induce a response in the symbiotic rhizobia. Flavonoids, which include is flavones, chalcones, flavonols, flavones, and anthocyanin amongst other related compounds, induce genes for nodulation in rhizobia to the host plants.

Activity of Growth Promoting Rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) include soil bacteria that colonize the roots of plants following inoculation of the seed and which enhance plant growth. These plant-growth promoting rhizobacteria (PGPR) have been identified as a biological control agent that could be an alternative to pesticide use for disease suppression without negative effects on the user, consumer, or the environment (Johnsson et al., 1998).

Key features of plant-growth-promoting rhizobacteria are their ability to colonize the root systems of plants and to modulate plant growth by enhancing the availability of nutrients. The rhizobacteria can also induce metabolic activities shift the phyto hormonal balance, induce defence mechanisms such as systemic acquired resistance (SAR) and induced systemic resistance (ISR), or by reducing phytotoxic microbial communities (Mc Cully, 2001).The mechanisms by which PGPR promote plant growth has been studied by several researchers including Mordukhova et al. (1991) who reported that rhizobacteria have the ability to produce or change the concentration of the plant hormones indole acetic acid (IAA), gibberellic acid, cytokinin, and ethylene (Arshad and Frankenberger, 1991; Glick et al*.,* 1995). The PGPR mechanism also performed a symbiotic N_2 fixation (Boddey and Do¨ bereiner,1995; Kennedy et al., 1997), antagonism against phyto pathogenic microorganisms e.g., *Fusarium* spp by production of siderophores (Scher and Baker, 1982), -1,3-glucanase, chitinases, antibiotics (Shanahan et al., 1992), cyanide and producing a solubilisation of mineral phosphates and other nutrients (De Freitas et al.,1997).

Biological control, i.e. the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of crop protection. Bio control involves harnessing disease-suppressive micro-organisms to improve plant health. Disease suppression by bio control agents involves sustained interactions among the plant, the pathogen, the bio control agent, the microbial community on and around the plant, and the physical environment (Handelsman and Stabb, 1996). Therefore, despite its potential in agricultural applications, bio control is one of the most poorly understood areas of plant-microbe interactions.

A bio control agent should grow and persist, or “colonize,” the surface of the plant it protects, and colonization is widely believed to be essential for bio control (Weller, 1983; de Wager et al., 1987; Parke, 1991). However, colonization, or even the initial size of the population of the bio-control agent, has been shown to be significantly correlated with disease suppression in only a few instances (Parke, 1991; Bull et al., 1991).

The use of plant-growth promoting rhizobacteria (PGPR) as inoculants to achieve various objectives

such as rapid early growth is a recent area of interest. Inoculants that promote rapid early seedling growth have potential to play an important role in agriculture, for example by promoting early growth of grass and legume pasture species. The establishment of pastures in acidic soils can be slow, resulting in greater weed competition and reduced opportunities to exploit soil resources. Opportunities for the use of PGPRs on wheat and other cereal crops are being investigated for similar reasons (Wakelin and Rider, 2004).

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Reference

Arshad, M. and Frankenberger, W.T. Jr. (1991). Microbe production of plant hormones. *Plant and Soil* **133**, 1-8.

Battacharyya, P.N. and Jha, D.K. 2012. Plant growth-promoting rhizobacteria (PGPR): the emergence in agriculture. *World Journal of Microbiology and Biotechnology* **28**, 1327-1350.

Benson, D.R. and Silvester, W.B. (1993). Biology of Frankia strains, Actinomycetes symbionts of actinorhizal plants. *Microbiology* **57**, 293–319.

Boddey, R.B., de Oliveira O. C., Urquiaga S., Reis, V. M., de Olivares, F. L., Baldani V. L. D., and Döbereiner, J. (1995). Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. *Plant and Soil* **174**, 195–209.

Boddey R. M., Giller, K. E., Cadisch, G., Alves, B. J. R., and Urquiaga S. (1998). Contribution of biological nitrogen fixation to tropical agriculture: actual and potential. *In* "Biological Nitrogen Fixation for the 21st Century" (C. Elmerich, A. Kondorosi and W E Newton, eds). pp. 599-604. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bladergroen, M. R. and Spaink, H. P. (1998). Genes and signals molecules involved in the rhizobia-leguminoseae symbiosis. *Current Opinions in Plant Biology* **1**, 353-59.

Boddey R.M., Urquiaga, S., Alves, B. J. R., and Reis, V. (2003). Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. *Plant and Soil* **252**, 139-149.

Bolan, N.S., M.J. Hedley, and White, R.E. (1991). Processes of soil acidification during Nitrogen cycling with emphasis on legume based pastures. *Plant and Soil* **134**, 53-63.

Bull, C.T., Weller, D.M., and Thomashow, L.S. (1991). Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. tritici by Pseudomonas fluorescence strain 2-79. *Phytopathology* **81**, 954-959.

Cohn J., Bradley, D. R., and Stacey G. (1998). Legume nodule morphogenesis. *Trends in Plant Science* **3**,105-110.

Dakora, F.D. (1995). Plant flavonoids: biological molecules for useful exploitation. *Australian Journal of Plant Physiology* **22,** 7-9.

Diagne, N., Arumugam, K., Ngom, M., Nambiar-Veetil, M., Franche, C., Narayanan, K. K., and Laplaze, L. (2013). Use of *Frankia* and Actinorhizal Plants for Degraded Lands Reclamation. *BioMed Research International* **2013**, 1-9. http://doi.org/10.1155/2013/948258

Franche, C., Laplaze, L., Duhoux, E., Bogusz, D. (1998). Actinorhizal symbioses: recent advances in plant molecular and genetic transformation studies. *Critical Review in Plant Sciences* **17**, 1-28.

Giller, K.E. and Wilson, K.J. (1991). "Nitrogen Fixation in Tropical Cropping Systems" pp 167-237. CAB International, Wallingford, England.

Gholami, A., Shahsavani, S., Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Biological Life Sciences* **1**, 3540-3583.

Glick, B.R., Karaturovic, D.M., and Newell, P. C. (1995). A novel procedure for rapid isolation of

plant growth promoting pseudomonads. *Canadian Journal of Microbiology* **41**, 533-536.

Handelsman, J. and Stabb, E.V. (1996). Bio control of soil borne plant pathogen. *American Society of Plant Physiologists* **8**, 1855-1859.

Hayashi, S., Reid, D.E., Lorenc, M.T., Stiller, J., Edwards, D., Gresshoff, P.M., Ferguson, B.J. 2012. Transient nod factor-dependent gene expression in the nodulation-competent zone of soybean (*Glycine max* L. Merr.) roots. *Plant Biotechnology Journal* **10**, 995-1010. doi: 10.1111/j.1467-7652.2012.00729.x.

Helyar, K.R. (1976). Nitrogen cycling and acidification. *Journal Australian Institute of Agricultural Science* **42**, 217-222.

Huss-Danell, K. (1997). Actinorhizal symbioses and their N fixation. *New Phytologist* **136**, 375-405.

Jensen, E.S. and Hauggaard-Nielsen, H. (2003). How can increased use of biological N_2 fixation in agriculture benefit the environment? *Plant and Soil* **252**, 177-186.

Johnsson, L., Hökeberg, M., and Gerhardson, B. (1998). Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against seed-borne diseases in field experiments. *European Journal of Plant Pathology* **104**, 701-711.

Kennedy, I.R., L.L Pereg-Gerk, C., Wood, R., Deaker, K. G., and Karupitiya, S. (1997). Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between Azospirilium and wheat. *Plant Sciences* **194**, 65-79.

Kloepper, J.W. and Schroth, M.N. (1978). Plant growth-promoting rhizobacteria on radishes. *In* "Proceeding of the 4th International Conference on Plant Pathogenic Bacteria 2", pp 879–882. Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France.

Kloepper, J.W., Leong J., Teintze, M. and Shroth, M.N. (1980). Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* **286**, 885.

Kraus, M., Fusseler, A., and Beck, E. (1987). *In situ* determination of the phosphate gradient around a root by radio autography of frozen soil sections. *Plant Soil* **97**, 407-418.

Ladha, J.K., Kamdu, D.K., Van Coppenolle, A. M.G., People, M.B., Canangal, V.R., Dart, P.J. (1996). Legume productivity and soil nitrogen dynamics in lowland rice-based cropping system. *Soil Science Society American Journal* **60**,183-192.

Ledgard, S. F. (2001). Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil* **228**, 43-59.

Leroug P., Roche P., Faucher C., Maillet F., Prome, J.C., and Denarie J. (1990). Symbiotic host specificity of *Rhizobium melilotiis* determined by a sulphate and acylated glucosamine oligosaccharide. *Nature* **344**, 781-784.

Lhuissier, F.G.P., de Ruijter, N.C.A., Sieberer, B.J., Esseling, J.J., and Emons, A.M.C. (2001). Time course of cell biological events evoked in legume root hairs by Rhizobium nod factors: state of the art. *Annual Botany* **87**, 289-302.

Parke, J.L. (1991). Root colonization by indigenous and introduced microorganisms. *In* "The Rhizosphere and Plant Growth" (D.L. Keister and P.B. Cregan, eds). Kluwer Academic Publishers.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., Van Der Putten, W.H. (2013).

Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11***, 789 - 799*

Prome, J. C., Denarie, J., and Truchet, G. (1998). Acylated chito oligomers are molecular signals that mediate the symbiotic interactions between nitrogen-fixing bacteria and their host plants. *Pure and Application Chemistry* **70**, 55-60.

Scher, F.M. and Baker, R. (1982). Effect of pseudomonas putida and a synthetic iron chelator wilt pathogens. *Phytopathology* **72**, 1567-1573.

Shahzad, S.M., Khalid, A., Arshad, M., and Rahman, K.U. (2010). Screening rhizobacteria containing ACC-deaminizes for growth promotion of chickpea seedling axenic conditions. *Soil and Environment* **29,** 38-46.

Shanahan, P., O' Sullivan, D.J., Simpsom, P., Glennon, J.D., and O'Gara, F. (1992). Isolation of 2.4-diacetylphlonaglucinol, a fluorescent pseudomad and investigation of physiological parameters influencing its production. *Applied Environment and Microbiology* **58,** 353-358.

Soltis, D.E., Soltis, P.S., Morgan, D.R., Swensen, M.R., Mullin, B.C. (1995). Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperm. *Proceeding National Academy of Science* **92**, 2647 – 2651.

Sprent, J.I. and Sprent, P. (1990). "Nitrogen Fixing Organism; Pure and Applied Aspects". 256 pp. Chapman and Hall, London.

Wakelin, S. A. and Ryder, M. H. (2004). Plant growth-promoting inoculants in Australian Agriculture. https://dl.sciencesocieties.org/publications/cm/abstacts/3/1/2004-0301-01-RV?access=0&view=pdf. [November 2015]

Wall, L.G. (2000). The actinorhizal symbiosis. *Journal of Plant Growth Regulation* **19**,167-182.

Weller, D.M. (1983). Colonization of wheat roots by a fluorescent *Pseudomonad* suppressive to take-all. *Phytopathology* **73**, 1548-1553.

Viprey, V., Perret, X., Broughton, W.J. (2000). Host-plant invasion by rhizobia. *Subcellular Biochemistry* **33**, 437-456.