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Review Paper

Rhizobia Unique Plant Growth Promoting Rhizobacteria: A Review

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Abstract

Rhizobia are well known nitrogen fixing bacteria. On the basis of generation time rhizobia is classify into slow (*Bradyrhizobium*) and fast growing Rhizobia (*Rhizobium*). Chemical fertilizer reduces the soil fertility and biofertilizer improve soil ecology. Rhizobia have some unique quality to fix nitrogen, produce plant growth hormones, Siderophore production, HCN production and good colonizer. Such activity directly or indirectly increases the plant growth and productivity. Co-inoculation of Rhizobia with other microorganism can also improve the plant growth and productivity. Biologiocal control activity enhances its important as PGPR. Few Rhizobia can tolerate few extend of salt, acidic environment, and low water content in soil. All above activity clearly indicate that Rhizobia are unique plant growth promoting rhizobacteria.

Key words: *Rhizobium, Bradyrhizobium, Siderophore, N_2-fixation, Biocontrol*

Introduction

Rhizobium- legume symbiosis is well known to fix Nitrogen. Biological nitrogen fixation is one of the effective methods to improve the plant growth and productivity. Shoko *et al*. (2007) mentioned that farmers can save nitrogen fertilizer by using vegetable soybeans and nitrogen saved was estimated at 80 kg nitrogen ha^{-1} as shown by the number of tillers, biomass and nitrogen in leaves of cane. Other scientist is also mentioned that substantial quantities of nitrogen symbiotically between 80 to 150 kg N ha^{-1} in 90 days (Toomsan, 1990; Deshwal *et al*., 2003a,b). Kernel nitrogen is either directly derived from nitrogen fixation as indicated by a maximized acetylene reducing activity at pod filling (Williams *et al*.,1990) or indirectly derived through metabolisation and translocation of plant nitrogen (Bray, 1983). All above information clearly indicates the importance of Biological nitrogen fixation.

Classification of Rhizobia

Rhizobia are nitrogen fixing bacteria and various classification system was applied for characterization of rhizobia. Hellriegel and Wilfarth, presented very convincing experimental reports in 1886 and 1888 to distinguish the innate ability of legumes of fix elemental nitrogen in the atmospheric nitrogen. Beijerinck isolated and cultivated a microorganism from the nodules of legumes in the year (1888). He named it as *Bacillus radiocicola* but Frank (1889) renamed it, *Rhizobium leguminosarum* (Fred *et al*., 1932) which is now placed in *Bergey's manual of determinative Bacteriology* (Holt *et al*., 1994).

(a) Fast and Slow Growing Rhizobia : Root nodulating bacteria have been differentiated on the basis of growth on defined substrate, as fast growers and slow growers (Löhis and Hansen, 1921). The slow growing bacteria have mean generation time greater than 6 hours and fast growing bacteria have less than 6 h in selective broth medium (Elkan, 1992). Most taxonomic reviews of the rhizobia state that the systematics of this family is in transition largely as a result of limited taxonomic research. There are about 750 genera of legumes containing 16000- 19000 species but only very few have been examined (Allen and Allen, 1981).

(b) Bergey's Manual of Determinative Bacteriology: When more and more bacteria were characterized morphologically, it become necessary to name and systematized them. Cohn (1872) convinced that the botanical species concept also applied to bacteria and this characterization was not to replace 1980's (see Table 1.1). *Bergey's Manual of Determinative Bacteriology*, was first published in 1923 in which cataloguing of information of identifying bacteria was included. Much revision, by the American Society for Bacteriology (now American Society for Microbiology) provided such references which were followed by *Bergey's Manual of systematic Bacteriology* (Holt *et al*., 1994).

Table 1.1: The first systematic scheme for bacteria (Cohn, 1872)

Division: Thallophyta
Order: *Schizosporeae*
Familia: *Bacteriaceae* (Schizomycetes)
Trilbus l: *Sphaerobacteria* (Kugelbakterien)

Genus 1: *Micrococcus*
Trilbus ll: *Microbacteria* (Stäbchenbakterien)
Genus 2: *Bacterium*
Trilbus lll: *Desmobacteria* (Fadenbakterien)
Genus 3: *Bacillus*
Genus 4: *Vibrio*
Trilbus lV: *Spirobacteria* (Schraubenbakterien)
Genus 5: *Sprillius*
Genus 6: *Spirochaete*

The concept of cross inoculating group, to define on the basis of their host range was challenged repeatedly by workers who found many examples of overlapping host range (Wilson, 1944). There was a class of strains that grow slowly with distinct properties. Jordan (1982, 1984) proposed *Bradyrhizobium* as a new genera (see Table 1.2) in the family Rhizobiaceae.

Table 1.2: Taxonomic classification of the rhizobia according to systematic bacteriology (Jordan 1982, 1984)

Recognized genera	Recognized species
Bradyrhizobium	*B. japonicum*
Rhizobium	*R. leguminosarum*
	R. leguminosarum bv. *trifolii*
	R. leguminosarum bv. *phaseoli*
	R. meliloti
	R. loti

In 1992 Elkan added two additional genera *Sinorhizobium* and *Azorhizobium* in their taxonomically classification (see Table 1.3).

Table 1.3; Taxonomic classification of the rhizobia according to Elkan (1992)

Recognized genera	Recognized species
Bradyrhizobium (Jordan, 1982)	*B. japonicum* (Jordan, 1982)
Rhizobium (Jordan, 1982)	*R. leguminosarum* (Jordan, 1982)
	R. meliloti (Jordan, 1982)
	R. loti (Jordan, 1982)
	R. galeage (Lindstrome, 1989)
	R. tropici (Martinz *et al.*, 1991)
	R. huakaii (Chen *et al.*, 1991)
Azorhizobium (Dreyfus *et al.*, 1988)	*A. caulinodans*
Sinorhizobium (Chen *et al.*, 1991)	*S. fredii* (Chen *et al*., 1991)
	S. xinjiangensis (Chen *et al*., 1988)

Although the existence of two distinct group of root nodule bacteria had been recognized since the early year of the last century (Fred *et al.*, 1932). The creation of the new genus *Bradyrhizobium* was the first accept change in rhizobial nomenclature (Jordan, 1982). *Bradyrhizobium* strain that nodulating soybean is characterized as *Bradyrhizobium japonicum* but this is not fit the definition of *B. elkanii*. This is the first recognize group of *Bradyrhizobium* strains (Young, 1996). *Bradyrhizobium elkanii* (Kuykendall *et al.*, 1992) has phenotypic and genetic characters which define a number of groups within the soybean nodulating bradyrhizobia. *Bradyrhizobium liaoningense* species has been defined for extra slow growing soybean rhizobia that form a coherent DNA-DNA hybridization group (Xu *et al.*, 1995). There are number of *Bradyrhizobium* strains that do not nodulate the soybean and no species names have yet been define for these, they are simply known as *Bradyrhizobium* sp. (Young, 1991). The most important single source of data for developing current classification of rhizobia, as of all other bacterial groups has been the sequencing of genes for 16s or small subunit of ribosomal RNA (SSU r RNA) (Jarvis *et al*., 1997). Four species in genra *Bradyrhizobium* are correctly recognized (see Table 1.4). Further, the new species shall not be given to *Bradyrhizobium* genus but host name shall be written in parenthesis as proposed by Young, 1991.

Table 1.4: Current list of rhizobial species based on Young *et al.* (2001)

Rhizobium	*R. leguminosarum*
	R. galeage (Lindstrome, 1989)
	R. tropici (Martinz *et al.*, 1991)
	R. etli (Segovia *et al.*, 1993)
	R. gallicum (Amargar *et al.*, 1997)
	R. giardini (Amargar *et al.*, 1997)
	R. lupini (Eckhart *et al*., 1931)
	R. phaseoli (Dangeared, 1926)
	R. mongolense (Van berkum *et al.*, 1998)
	R. undicola (Young *et al.*, 2001)
	R. hainanensis (Young *et al*., 2001)

	R. hautlense (Wang *et al.*, 1998)
	R. radiobacter (Young *et al.*, 2001)
	R. rhizogenes (Young *et al.*,2001)
	R. rubi (Young *et al.*, 2001)
	R. vitis (Young *et al.*, 2001)
	R. yanglingense (Tan *et al.*, 2001)
Bradyrhizobium	*B. japonicum* (Jordan, 1982)
	B. elkanii (kuykendall *et al*., 1992)
	B. liaoningense (Xu *et al.*, 1995)
	Bradyrhizobium sp. (Young *et al.*, 1991)
Mesorhizobium	*M. loti* (Jarvis *et al.*, 1997)
	M. huakuii (Jarvis *et al*., 1997)
	M. ciceri (Jarvis *et al.*, 1997)
	M. tianshanense (Jarvis *et al.*, 1997)
	M. mediterraneum (Jarvis *et al.*, 1997)
Azorhizobium	*A. caulindans* (Dreyfus *et al.*, 1988)
Sinorhizobium	*S. fredii* (Chen *et al*., 1988)
	S. indiaensis (Young *et al*., 2001)
	S. kostiense (Young *et al*., 2001)
	S. meliloti (De Lajudie *et al.*,1994)
	S. morelense (Young *et al.*, 2001)
	S. saheli (Young *et al.*, 2001)
	S. teranga (Young *et al.*, 2001)
	S. xinjiangensis (Chen *et al.*, 1998)
	S. medicae (Rome *et al.*, 1996)
	S. abri (Young *et al*., 2001)
	S. arboris (Young *et al*., 2001)

Negative impact of chemical fertilizer on agriculture

As an intensive farming is expanded to increase agricultural productivity, the increased in the use of chemical inputs such as fertilizers and pesticide have increased water and soil pollution and increased the deterioration of ecological system (Kim and Stoecker, 2006). When natural fossil fuels finished up, ultimately the present practices of industrial production of N-fertilizer will suffer. Thus, the fuel energy is directly related with crop productivity. As the productivity declines food and feeding will be affected. To stop such a disaster, legumes- *Rhizobium* biotechnology may properly be modulated to meet the demand of crop productivity on sustainable basis. Extensive use of chemicals has disturbed the ecological balance of soil, pollute the ground water, create resistant races of pathogen and health risk to men (Loper and Ishimaru, 1991). Ayala and Rao (2002) concluded that higher dose of chemical fertilizer disturb microbial ecology and decrease soil fertility. Previously, Bhattacharya and Roy (2000) observed that chemical fertilizer inhibited the growth of rhizobia.

What is Biofertilizer and positive impact on agriculture?

The term “Biofertilizer” can generally be defined as preparation containing live or latent cells of efficient strains of Nitrogen fixing, Phosphate solublising or cellulolytic microorganisms used for application to seeds, soil or composting areas with the objective of increasing the number of such microorganisms and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants (Boraste *et al.*, 2009).

A global inventory of the process of nitrogen for agriculture crop production indicated that biological nitrogen fixation is predominant; approximately 175 million metric tones per year of nitrogen (gaseous) is fixed biologically (Burns and Hardy, 1975). Brockwell and Bottomely (1995) concluded that particular N_2 fixation by legumes, an ecologically efficient substitute for fertilization of crops and pasture with inorganic N. Biological nitrogen fixation (BNF) contribute to productivity both direct, where the fixed nitrogen is harvested in grain or other food for human or animal consumption, or indirectly by contributing to sustain or induce soil fertility in the agricultural system by adding N to the soil. Economically important legumes plants are inoculated with the effective strains of rhizobia mixing with certain carrier materials (Smith, 1992). The nature and quality of carrier material often determine the subsequent performance of the inoculant (Smith, 1995). The term “legume inoculant” means the application of specific rhizobial cell to seeds or directly to plant at their seedling stage for proper growth of the plants. Many formulation and methods for application have been reported and proposed to commercialization (Bashan, 1998). New and creative inoculants may assist in improving the introduction of legume inoculants so as to increase the quantity and utilization of biological fixed nitrogen. Deshwal *et al.* (2003b) mentioned that Rhizobia promote the growth of plants either directly through N_2 fixation, supply of nutrients, synthesis of phytohormones and solubilization of minerals, or indirectly as a biocontrol agent by inhibiting the growth of pathogens. The biocontrol effect of rhizobia is due to the secretion of secondary metabolites such as antibiotics and HCN. Siderophore production in iron stress conditions provides rhizobia an added advantage, resulting in exclusion of pathogens due to iron starvation.

Growth Promoting Rhizobacteria (PGPR)

Rhizobia strains improve plant growth and productivity by production of various chemicals in rhizosphere. These chemicals are plant growth hormones, siderophore, phosphorous solubilization and production of HCN. Various observations have been observed by various researchers. Deshwal *et al.* (2003a) screened the plant growth promoting *Bradyrhizobium* (*Arachis*) sp. in peanut which produced siderophore and IAA, and exhibited phosphate solubilization *in vitro*. Deshwal *et al.* (2003a) mentioned that Peanut seeds coated with *Bradyrhizobium* strains were significant by enhanced seed germination, seedling biomass, nodule number, nodule fresh weight, average nodule weight. Plant growth promoting bacteria are the natural potential resource which colonize roots of plants and stimulates growth and yield directly and indirectly (Afzal and Bano, 2008). Mia et al (2012). Recently, legume bacteria (*Rhizobium* spp.) have been considered as a PGPR for legume as well as non-legumes and have the potential for growth stimulation. PGPR activity of rhizobia as follows.

(a) Nitorgen fixation: The symbiotic interactions between a legume and rhizobia result in a unique, nitrogen fixating plant organ, the nodule symbiotic nitrogen fixation throughout nodulation in legumes is well known, which help to reduce the application of inorganic N and can also play a major role as green manure in improving the soil fertility (Sanginga *et al.,* 1996; Bellone *et al.*, 1997). According to Postgate (1982), the atmosphere contains about 10^{15} tones of N_2 gas and nitrogen cycle involves the transformation of some 3 x 10^9 tonnes on global basis but lighting can fix atmospheric nitrogen i.e. transformation and 10% of world supply of nitrogen meets out by this process (Sprent and Sprent, 1990). The fertilizer industry also provides chemically fixed nitrogen globally. The consumption of fertilizer N increased from 8 to 17 Kg. ha^{-1} of agriculture land in the 15-year period from 1973 to 1988 (FAO, 1990).

More than 100 years biological nitrogen fixation (BNF) has commanded the attention of scientists concerned with plant mineral nutrition and it has been exploited extensively in agricultural field (Dixon and Wheeler, 1986; Burris, 1994). Frages (1992) suggested that ecological principles and practices that are appropriate for the manipulation of rhizobia prove suitable model for other soil microorganism as well. Brockwell and Bottomley (1995) concluded that in particular efficient substitute for fertilization of crops and pastures occurs with the organic N.

(b) Phosphorous solubilizing bacteria: Phosphorous is essential element for plant growth and productivity. Few rhizobial strains has capability to solubilize non-solubilizing phosphorous in soil and as a results increase plant growth and productivity. Halder and Charkraborty (1993) reported that a large number of *Bradyrhizobium* strains are able to solubilize inorganic phosphate. In field study, Chabot *et al*. (1996) observed that phosphate solubilization by strains of *R. leguminosarum* bv. *phaseoli*, was the most important mechanism of maize and lettuce growth promotion. Antoun *et al*. (1998) also found that *Bradyrhizobium* sp (Lupinus) solubilized phosphate. Similarly, Dashi *et al.*, (1998) observed that plant growth promoting rhizobacteria solubilized phosphate and accelerate nodulation, increase nitrogen fixation activity by field grown *Glycine max* L. Merr. under short season conditions.

(c) Plant Growth hormones: The symbiotic bradyrhizobia enhance plant growth by production of plant hormones like IAA (Tien *et al.*, 1979; Hussain *et al.*,1987), IAA is well-known plant growth promoting hormones and 96% symbiotic nitrogen fixing rhizobia produced IAA (Prevost *et al*., 1987; Arora *et al.*, 2001). Lippman *et al.* (1995) observed that PGPR could directly enhance plant growth by IAA production and increasing nutrient uptake. Noel *et al*. (1996) observed under gnotobiotics conditions, a direct growth promotion of the early seedling root, appears to involve the growth regulators such as IAA and cytokinin.

(d) HCN production: One of the reasons of inhibition is production of cyanogens by PGPR. PGPR produced HCN to control growth of different type of pathogen (Bagnasco *et al.*, 1998). Available literature revealed that rhizobia also produced HCN. Beauchamp *et al* (1991) found that 4 out of 32 rhizobial strains produced HCN production. Alvarez *et al.* (1995) observed that less than 1% rhizobia isolated from tomato rhizosphere showed positive for HCN production. Nautiyal (1997) screened *Rhizobium* strains, among isolated 256 bacterial strains *Rhizobium* NBRI 19513 completely inhibited growth of *Fusarium oxysporum*, *Rhizoctonia bataticola* and *Pythium* sp. *in vitro*. Antoun *et al* (1998) observed that 3% *Rhizobium* and *Bradyrhizobium* strains were produced HCN. The HCN producing rhizobia or bradyrhizobia inhibited the plant growth of soil borne pathogens but also these symbiotic bradyrhizobia and rhizobia produced some toxin, which inhibited the growth of fungi, Chakraborty and Purkayastha (1984) observed that quantity of rhizobitoxine was significantly decreased the growth of *M. phaseolina*. *Bradyrhizobium* elkanii accumulated rhizobiotoxine in culture and in nodules (Devine *et al*., 1988; Kuykendall *et al.*, 1992). The rhizobitoxine has positive effect on nodulation, the inconsistency can be explained by difference in the sensitive of nodulation among leguminous species (Hunter, 1993; Xie *et al.*, 1996; Schmidt *et al*., 1999), as also evidenced by the work of Duodu *et al.* (1999) who stated that rhizobitoxine is an important compound involved in symbiosis between rhizobia and legumes. Rhizobitoxine is an ethylene synthesis inhibitor that is produced by legumes symbiont *Bradyrhizobium* (Xie *et al.*, 1996). Deshwal *et al.*, (2003a) observed that *Bradyrhizobium* strains AHR-2amp+, AHR-5amp+ and AHR-6amp+ showed antagonistic activity against *Macrophomina phaseolina.*

(e) Siderophore prodcution: Siderophore production has been reported in various species of root nodulating bacteria such in fast growing *Rhizobium* spp (Carson *et al*., 2000; Arora *et al.*, 2001) and *Bradyrhizobium* (Deshwal *et al.*, 2003a). Root nodulating rhizobia produce a number of siderophores but only some siderophores have been structurally characterized, these include carboxylates such as rhizobactin from *Sinorhizobium meliloti* (Smith *et al*., 1985), catechol from *Bradyrhizobium* (cowpea) (Modi

et al., 1985), anthranilate from *Rhizobium leguminosarum* biovar *viciae* (Rioux *et al*., 1986). catechol from *Bradyrhizobium* (Peanut) (Nambiar and Sivaramakrishnan, 1987), *Rhizobium leguminosarum* (Patel *et al*., 1988), *Rhizobium leguminosarum* biovar *trifolii* (Skorupska *et al*., 1989), citrate from *Bradyrhizobium japonicum* (Guerinot *et al*., 1990), dihydroxamate rhizobactin 1021 from *Sinorhizobium meliloti* (Persmark *et al*., 1993), vicibactin, a trihydroxamate from *Rhizobium leguminosarum* biovar *viciae* (Dilworth *et al*., 1998).

Bio-control activity of rhizobia against certain pathogens

A large number of fungicides have been used to control pathogenic fungi but safer alternative are needed to protect the environment by beneficial, microorganisms. Hanshem *et al*., (1997) reported that most fungicides affected survival of *Bradyrhizobium* inoculants on peanut seed and resistant bradyrhizobia survived more than sensitive bradyrhizobia. Green house and field tests indicated that *Bradyrhizobium* have variation in fungicide tolerance, peanut *Bradyrhizobium* strains from different peanut cultivars have been previously reported (Aliwi *et al*., 1989; Taber *et al*., 1994).

Biocontrol research has gained considerable attention and appears promising as a viable alternative to chemical control strategies (Rebafka *et al*., 1993). Actually, biological control is a nonhazardous strategy to reduce crop damage caused by plant pathogens (Cook *et al*., 1995). The control of soil-borne pathogens with antagonistic bacteria has been intensively investigated (Chakraborty and Purkayastha, 1984; Enteshamul and Graffer, 1993; Paulitz and Fernand, 1996). Root colonization is an important first step in interaction of beneficial bacteria with plant (Chao, 1990; Kloepper and Beauchamp, 1992). Rhizosphere resident antagonistic microorganisms are ideal biocontrol agents, as the rhizosphere provides the front-line defiance for roots against infection by the soil borne pathogens (Lumsden *et al*., 1995).

Similarly Buonasissi *et al*. (1986) reported that some strains of *Rhizobium phaseoli* inhibited mycelial growth of *Fusarium* spp. *In* vitro.Few strains of rhizobia are reported to inhibit sclerotia germination of *Sclerotium rolfsii* (Balasundaram and Sarbhoy, 1988) and colony growth of *Phytophthora megasperma* (Tu, 1978), *Macrophomina phaseolina* (Chakraborty and Purkayastha, 1984; Arora *et al*., 2001). *Bradyrhizobium* bacterized seeds are known to have reduced *Macrophomina phaseolina* infection (Enteshamul and Graffar, 1993), Malajczuk *et al*. (1984) isolated the rhizobia from the root nodules of *Acacia pulchella* and concluded that rhizobia with other microbes inhibit *Phytophthora cinnamoni* zoospore *in vitro*. Chao (1990) studied the antifungal activity of *R. leguminosarum*, *R. meliloti* and *Bradyrhizobium* against *Trichoderma harzianum*, *Pythium ultimum*, *Fusarium oxysporum*, *F. solani* and concluded that rhizobia or bradyrhizobia not only fix nitrogen but also have an ability to control the pathogenic fungi. Sixty four strains of *Rhizobium* were evaluated by streak-plate and spent culture method to determine their antifungus activity against *Macrophomina phaseolina*. They were found that rhizobia inhibited the growth of *Macrophomina phaseolina* as also observed by Perdomo *et al*. (1995). Deshwal *et al*., (2003a) observed that bradyrhizobial strains controlled *Macrophomina phaseolina* infection *in vitro* as well as *in vivo* and reported that *Bradyrhizobium* enhanced seed germination, nodule number and grain yield in peanut. Previously, Arora *et al*. (2001) also observed that similar observation when *R. meliloti* introduced in *M. phaseolina* infected soil reduction in number of *M. phaseolina* as well enhanced growth of peanut.

Effect of environmental factors on rhizobia

Much attention has been paid to the focus on the factors that affect the ability to establish inoculant strains on plants growing in soil with indigenous rhizobia. Different type of environmental factors reported to affect competition of nodulation such as presence of indigenous rhizobia (Thies *et al*., 1991), soil temperature and type of soil (Ham *et al*., 1971), soil moisture contents (Boonkerd and Weaver, 1982), soil pH (Dughn and Bottomley, 1984), nitrogen availability (Abaidoo *et al*., 1990), microbial antagonists (Deshwal, 2003a).

(a) Salt Tolerance: Generally the root nodulating *Bradyrhizobium* and *Rhizobium* spp. are more salt tolerance than their host legumes; they showed marked variation in salt tolerance. EI-Sheikh and Wood (1990) isolated soybean and chickpea rhizobia, tolerant to 340mM NaCl and concluded that fast growing being more tolerant than slowing strains. Salinity is a serious threat to agriculture in arid and semi-arid regions (Rao and Sharma, 1995). Increase salt concentrations in soil and have detrimental effects on soil microbial populations as a result of direct toxicity as well as through osmotic stress (Tate, 1995). Salinity decreased plant growth and yield, depending upon the plant species and salinity level (Delgado *et al*., 1994). Some legumes like *Phaseolus vulgaris*, *Glycine max* are more soil tolerant than other legumes like *Pisum sativum* and it has been reported that *Vicia faba* tolerant lines sustained nitrogen fixation under saline conditions (Cordovilla *et al*., 1995). *Rhizobium* from woody legumes (*Acacia* spp.) also showed substantial salt tolerance strains to the extent of 500 to 850mM NaCl (Lal and Khanna, 1995). Most of the rhizobia due to their sensitive to sodic soil environment adversely affect the nitrogen fixation capacity resulting in the loss of productivity of legumes (Peoples *et al*., 1995; Idrissi *et al*., 1996). Arora *et al*. (2000) observed that the *R. meliloti* isolated from *Mucuna pruriens* tolerate salinity stress up to 950 mM *in vitro*.

Le Rudulier and Bernard (1986) already identified intracellular accumulation of glycine betaine in rhizobia and it's concentration is more in the salt tolerant strains of *R. meliloti* as compared to sensitive strains of *R. melilotii* (Fougere and Rudulier, 1990). When rhizobia are growing under salt or osmotic stress, the disaccharide trehalose play an important role in osmoregulation (EI-Sheikh and Wood, 1990) and trehalose accumulates to higher level in cells of peanut rhizobia (Ghittoni and Bueno, 1996). Previous reports of Ghittoni and Bueno (1995) stated that slow growing rhizobia of peanut accumulate trehalose when mannitol acted as carbon source *in vitro*. Other carbohydrate like sucrose and ectoine were used as osmoprotectant for *Sinorhizobium meliloti* (Gouffi *et al*., 1999).

It is generally believed that salt sensitive rhizobia are less beneficial than salt resistance but El-Sheikh and wood (1995) evaluated the effect of soybean nodulation in soil. They found that no significant difference observed in shoot and root dry weight, nodule number and nodule weight between salt-tolerant and salt sensitive strains, N_2 fixation was less in sensitive than salt tolerant strains.

(b) Low moisture level: Rhizobia can exist in soil with limiting moisture levels. Jenkins *et al.* (1989) observed that slow and fast growing rhizobia established effective nodulation in legumes growing in desert soil. Water stress eventually led to a reduction in infection and nodulation in legumes (Hunt *et al.*, 1981). Symbiotic N_2 fixation of legumes is also highly sensitive to soil water deficiency in such legumes like *Pisum sativum* (Abdel-Wahab and Zahran, 1979), *Medicago sativa* (Abdel-wahab and Zahran, 1983), *Vicia faba* (Guerin *et al*., 1990), *Vigna* (Pararjasingham and Knievel, 1990) and *Arachis hypogaea* (Simpson and Daft, 1991).

(c) High temperature: High soil temperatures in tropical and subtropical area are a major problem for biological nitrogen fixation of legumes like peanut (Kishinevsky *et al.*, 1992) as critical temperature for N_2 fixation is 40^{o}C for peanut (Michiels *et al*., 1994).

(d) Acidic soil: Bradyrhizobia have generally been considered higher tolerance to acid pH than that of fast growing strains (Graham *et al*., 1980) but it has been observed that few *Bradyrhizobium* strains failed to grown at pH 4.5 (Cooper *et al*., 1985). Rossum *et al.* (1994) observed that some *Bradyrhizobium* strains performed symbiotic relationship in groundnut crop under acidic stress (pH 5 to 6.5) while other performed ineffective under such conditions so there is variation in similar group of *Bradyrhizobium* strains to tolerance towards acidic pH level.

(e) Micronutrient and Macronutrient iron: Like other micronutrient and macronutrient iron is also necessary for living organism. Iron is the fourth most abundant element in the earth. Iron oxides, comprising minerals such as hematite, magnetite and limonite are most abundant of metal oxides in soil (Schwertmann and Taylor, 1989). Iron is required for large variety of metabolic process in virtually all organisms (Crichton *et al*., 1987) except *Lactobacilli* (Archibald, 1983). In aerobic condition, iron is present in soil of neutral pH as insoluble ferric hydroxide polymers are not available biologically (Linhsay, 1979). Most microorganisms have efficient high affinity iron uptake system, to fulfill their requirements. In this process siderophore; low molecular weight iron (III) chelating agents are synthesized (Neilands, 1981). Siderophores chelate insoluble iron and solubilize iron and ferric siderophore complex are taken up by the cell through specific membrane receptors (Neilands, 1982). Tang *et al.* (1990) observed that number of nodule initials were depressed when the iron concentration was low but when initiation had occurred nodules developed normally *in vitro.*

Rhizobium bio-formulation

Rhizobia have plant growth promoting activity. People are very much concern about the Rhizobia as biofertilizer. For evaluating the potency and efficacy of rhizobia in field, it is necessary to select the good carrier material. Characteristics of an excellent carrier the based on the properties e.g. high water holding capacity, high water retention, nearly sterile, nontoxic, biodegradable, no heat on wetting, uniform physically as well as chemically, having bufferering capacity, including in rhizobial population and survival, easily available, reasonable cost and tolerant towards adverse environmental conditions are desirable characteristics (Smith, 1992; Trevors *et al.*, 1992). Hot and dry conditions at the time of planting cause rapid decrease in rhizobia on bacterized seed. Rhizobial inoculant would take at least US$ 87 worth of urea to produce a soybean yield comparable to that possible only US$3 worth of rhizobial inoculant (Somasegeran *et al*., 1992). Kremer and Peterson (1983) successfully applied the oil-based peat, *Rhizobium* inoculants coated on seeds of bean, peanut and cowpea. They observed that bio-inoculant increased nodulation, fresh weight yield in all crops Types of carrier material also play an important role in inoculant formulation because carrier create suitable environment as well as provide moisture and nutrient value. Although, in the western countries, peat is the most commonly used carrier of rhizobia inoculants but being tropical country, the lack of suitable local peat in many areas of world including India leads to interest in other material like charcoal, vermiculite, peanut hulls, corn cobs, saw mill waste, lignite etc. Among them only peat, charcoal, vermiculite and lignite were successfully used carriers whereas peanut hulls, corn cobs are unsatisfactory (Sparrow and Ham, 1983; Somasegeran *et al.*, 1992).

Carrier material may act to enhance survival of inocula by promoting microorganisms with a protective environment in order to escape unfavorable conditions in soil. The reasons for a decrease in microbial inoculum populations in soil over time include insufficient nutrients available for maintenance and replication and sub optimal environmental conditions (Van Elsas and van Overbeek, 1993). The most widely used carrier for legumes inoculant are natural compounds such as peat, soil, compost and plant material (Thompson, 1980; Chao and Alexander, 1984; Smith, 1995; Richter *et al.*, 1989; van Veen *et al.*, 1997). Kostov and Lynch (1998) used composted sawdust as possible carrier for *Bradyrhizobium* and observed that composted sawdust (carrier material) supported the growth of *Bradyrhizobium*.

Good root colonization is indicator of improve plant growth: Inoculant is required if sufficient number of effective rhizobia are not present in soil. However, it is not always easy to predict their presence, although there are several indicators based on previous land management. Soil often contains rhizobia that are highly competitive against those applied in inoculants. Selective strains of peanut *Rhizobium* failed to increase yield in the presence of high population of indigenous rhizobia (Vander Merwe *et al*., 1974). Inoculant available commercially in worldwide vary in number and effectiveness. The key being that no inoculant strain can perform successfully unless the rhizobia it contains are viable and form an appreciable quantities of effective nodules on

desired plants. Strain, number of rhizobia and type of carrier can account for wide variations in effectiveness of different inoculants (Hiltbold *et al.*, 1980). Import of inoculant has it's own difficulty for often as by the time inoculants arrive in the country and are distributed among the growers, the viability of inoculants decrease resulting into loss of beneficial properties.

Application of PGPR better then Chemical fertilizer

Due to non-encouraging results after continuous use of chemical fertilizer, use of biological agents, such as PGPR proved beneficial in comparison to that of chemical fertilizers. Hoque (1993) used *Bradyrhizobium* inoculant as a source of N nutrition for grain legumes. The inoculants markedly increased nodule number, nodule mass, shoot weight and crop yield in comparison to urea treated plants. In the other crop like groundnut, lentil and mung bean was also increased grain yield 30-47% were obtained as compared to control and 13-7 % over urea treatment. Several workers observed root hair curling and induced plant growth in non-legumes like maize, rice and oat (Terouchi and Syong, 1990). Some *Bradyrhizobium* sp. could form nitrogen fixing nodules with *Parasponia* a nonlegume (Werner, 1992). Nod factors produced by *Bradyrhizobium* reported to induce alkalinization in tomato cell cultures (Staehelin *et al.*, 1994). Nodule like structures formed by rhizobia have been observed in oil seed crop (*Brassica napus*) by Trinick and Hadobas (1995). Wiehe and Höflich (1995) demonstrated that rhizobia can mulitiply and survive under field conditions, in the rhizosphere of non-host legumes. All other slow growing strains were assigned to the *Bradyrhizobium* spp., the so-called cowpea miscellany rhizobia or tropical bradyrhizobia, to false concepts about the ubiquity of tropical bradyrhizobia, the promiscuity of tropical legumes and the inaccurate recommendation of inoculants (Thies *et al*., 1991). Rhizobia can influence yield due to their potential to establish nodulation capacity and effectiveness in symbiotic nitrogen fixation. Seed yield increased in groundnut due to in response of *Bradyrhizobium* strains the field trails (Nambiar, 1985). On the other-hand, Rebafka *et al.* (1993) carried out field experiments successively for three years on an acid sandy soil, to asses the effect of millet straw application on growth and N_2 fixation of groundnut and found that 3 year of crop residue (4 ton ha^{-1} yr^{-1}) increased symbiotic nitrogen fixation, total dry matter production (haulm plus pods) by 83% and total nitrogen (N) accumulation by 100%. Simanungkalit *et al.* (1995) observed that *Bradyrhizobium* strains enhanced the nodulation and grain yield in soybean in acidic soil. Further study root colonization in soybean rhizosphere by introduced strain successfully colonized the root resulted enhance nodulation.

Bradyrhizobium sp. strains enhanced seed germination, nodule number, shoot weight, N content in peanut plant. Commercial bio-inoculants failed to have significant yield response when compared with local bradyrhizobial strains (Olufajo and Adu, 1993). Okereke *et al.* (2000) observed that introduced strains USDA 110 occupied the higher percentage of nodule sites because it was more competitive than the other *Bradyrhizobium* strains and introduced strain enhanced shoot dry weight, percentage N, total N, nodulation and seed yield.

(a) Siderophore effectively control the disease chlorosis: The mechanism by which plant avoid iron (**chlorosis**) are both more diverse and less investigated than the siderophore mediated iron up take system of microorganisms. Three strategies of iron assimilation have been identified in plant (Bienfait, 1989). Strategy I, found in non-graminaceous monocots and all dicots, involves acidification of the rhizosphere, thus increasing iron solubility by approximately 10^3 per pH unit, the reduction of Fe^{3+} ion and Fe (III) chelates to Fe^{2+} ion and uptake of Fe (II) occurs. Strategy II, observed that in graminaceous monocots secretion of iron chelating agent (phytosiderophore) of mugineic acid family involves (Sugiura *et al.*, 1981) and where as in strategy III, was the uptake of microbial Fe (III) siderophores take place. Although extensive research has been directed to correct chlorosis by the applications of available iron compounds to the soil. Hence the, the siderophores production by rhizobial strains would prove considered as a potential way to improve nodulation and N_2 fixation in iron deficient conditions (Carson *et al.*, 1992).

(b) Co-inoculation and Plant growth: Co-inoculation of *Azospirillium* with *Bradyrhizobium* enhanced nodulation and root growth of soybean (Molla *et al*., 2001). Earlier also several encouraging but inconsistent results on nodulation and N_2 fixation have been reported in different legumes in co-inoculation studies of *Azospirillium* and *Bradyrhizobium* (Sarig *et al.*, 1986; Del Gallo and Fabbri, 1991; Burdman *et al.*, 1997). Antoun *et al.* (1998) observed that symbiotic *Bradyrhizobium* strain enhanced 15% yield of radish and concluded that specific bradyrhizobia have the potential to be used as plant growth promoting rhizobacteria (PGPR).

Rice *et al.* (1994) suggested that mixed culture of *Rhizobium meliloti* and phosphorous solubilizing fungi *Penicillium bilaii* were more efficient because fungus solubilized phosphorous and, the later was taken up by plant whereas *Rhizobium* enhanced nodulation as well as nitrogen fixation.

(c) Colonization: Successful colonization of the legume rhizosphere by an inoculum strain of *Rhizobium* or *Bradyrhizobium* showed that these bacteria compete effectively with the indigenous microorganisms due to the utilization of the by using the organic compounds excreted by the root (Vander Merwe *et al.*, 1974). These indigenous species are numerous and many grow rapidly but root nodulating bacteria like *Bradyrhizobium* grow very slowly. Despite the slow growth they have, as a result of their capacity to nodulate and together with the host legume, bring about the fixation of N_2. As a matter of fact, Anderson (1957) noted that the number of nodules formed by *Rhizobium leguminosarum* biovar *trifolii* was reduced in *Trifolium repens* L. and were prevented by several bacteria that did not produced antibiotics, similarly Plazinski and Rolfe (1985) reported that strains of *Azospirillium* that do not produce antibiotics were found to reduce nodulation of in *Trifolium subterrancum* and *T. repens*. The antibiotics producing bacterial strains suppress many of the indigenous bacteria and proliferate more extensively in the rhizosphere because of the reduced competition (Li and Alexander, 1988).

Conclusion

Chemical fertilizer disturbs soil ecosystem so there is need to screen and find out the alternative of chemical fertilizer. Plant growth promoting rhizobacteria is good choice. Rhizobia are symbiotic nitrogen fixer and effectively colonize in plant rhizosphere. Rhizobia strains produce plant growth hormones, solubilize phosphorus, siderophore production, HCN etc in symbiotic as well as free living form. Further, few rhizobial strains can inhibits the growth of pathogenic fungus. Rhizobia effectively colonize with other microorganism. Literature suggests that few strains of rhizobia can survive under some extend of unfavorable condition in soil. Bio-inoculants of rhizobia strains are effectively improve the plant growth and productivity. All above information suggests that rhizobia are unique plant growth promoting rhizobacteria.

References

Shoko, M.D.; Tagwira, F. and Zhou, M. (2007) The potential of reducing nitrogen fertilizer rates using a soyabean-sugarcane production system in the South Eastern Lowveld of Zimbabwe. Afr. J. Agri. Res. 2 (9): 475-480.

Toomsan, B. 1990 Groundnut Microbiology Research at Khon-Kamen Univ. In, *Groundnut improvement, report* of work for 1986-88.) ed. A. Patanothai.. Khoankhen University, Thailand), pp 89-110.

Deshwal, V.K.; Dubey, R.C. and Maheshwari, D.K, (2003a) Isolation of plant growth promoting strains of *Bradyrhizobium Arachis* sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut. Curr. Sci., 84(3): 443-448.

Deshwal, V.K.; Pandey, P.; Kang, S.C. and Maheshwari, D.K. (2003b) Rhizobia as a biological control agent against soil borne plant pathogenic fungi. Ind. J. Exp. Biol. 41: 1160-1164.

Williams, J.G.K.; Kunelik, A.P.; Livak, K.J.; Rafalski, J.A. and Terry, S.V. 1990 DNA polymorphism amplified by arbitrary primer and used as genetic markers. Nuclic Acid Res. 18: 6331-6335.

Bray, C.M. (1983) Nitrogen metabolism in plants.eds Longman group Ltd, London. pp, 214.

Hellriegel, H. and Wilfarth, H. (1988) Untersuchungen über die stickstoffnahrung der Gramineen und Leguminosen. Belageheft zu der zeitschrift des zeitschrift des vereins Rübenzucker-Industrie Deutschen, Reiches 1: 234.

Beijerinck, M. W. (1988) Die Bacterian der Papillionaceenknollchen. Bot. Ztg. 46:726-735.

Frank, B. (1889) Uber die Pilzsymbiose der Leguminosen. Berichte der Deutschen Botanischen Gesellschaft 7: 332-346.

Fred, E.B.; Baldwin, I.L. and McCoy, E. (1932) Root nodule bacteria and leguminous plants. University of Wisconsin Studies in Science No.5. University of Wisconsin, Madison.

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994) In, Bergey's manual of Determinative Bacteriology. Williams and Wilkins Press, Baltimore, USA.

Löhis F. and Hansen R. (1921) Nodulating bacteria of leguminous plant. J. Agric. Res. 20: 543-556.

Elkan, G.H. (1992) Taxonomy of the rhizobia. Can. J. Microbiol. 38: 446-450.

Allen, O.N. and Allen, E.K. (1981) The Leguminosae. University of Wisconsin Press, Madison, Wis.

Cohn, F. (1872) Untersuchungen ueber Bacterien. Beitr. Biol. Pflanz. Heft. 21: 127-224.

Wilson, J.K. (1944). Over five hundred reason for abandoning the cross inoculation groups of legumes. Soil Sci. 58: 61-69.

Jordan, D.C. (1982) Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov. a genus of slow growing root nodule bacteria from leguminous plants. Int. J. Sys. Bact. 32: 136-139.

Jordon, D.C. (1984) Family II. *Rhizobiaceae.* In , *Bergey's manual of systematic bacteriology*. vol. I (eds. By N. R. Krieg and J. G. Holt Williams and Wilkins Co., Baltimore, M .D): pp 232-242.

Young, J.A.W. and Haukka, K. (1996) Diversity and phylogeny of rhizobia. New. Phytol. 133: 87-94.

Kuykendall, L.D.; Saxena, B.; Devine, T.E. and Udell, S.E. (1992) Genetic diversity in *Bradrhiozbium japonicum* Jordan. 1982 and a proposal for *Bradyrhizobium elkani* sp. nov. Can. J. Microbiol. 38: 501-505.

Xu, L.M.; Ge, C.; Cui, Z.; Li, J. and Fan, H. (1995) *Bradyrhizobium liaoningensis* sp. nov. isolated from the root nodules of soybean. Int. J. Sys. Bacteriol. 45: 706-711.

Young, J.P.W. (1991) Phylogenetic classification of nitrogen-fixing organisms. In Biological nitrogen Fixation (eds Stacey, G. Burris and Evans H.J., Chapman and Hall, New York, USA.): pp. 43-86.

Jarvis, B.D.W.; van Berkum, P.; Chen, W.X.; Nour, S.M.; Fernandez, M.P.; Cleyet-Marel, J.C. and Gillis, M. (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum* and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int. J. Sys. Bacteriol. 47: 895-898.

Young, J.M.; Kuykendall, L.D.; Marinez-Romero, F.; Kerr, A. and Sawada, H. (2001) A revision of *Rhizobium* frank, 1889, with an emended description of the genus and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajude, et. al. 1998 as new combinations, *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*. Int. J. Sys. Evol. Microbiol. 51: 89-100.

Kim, C-G and Stoecker, A. (2006) Economic Effects of Environmental Taxation on Chemical Fertilizers. International Association of Agricultural Economists Conference, Gold Coast, Australia, August 12-18, 2006.

Loper, J.E. and Ishimaru, C.A. (1991). Facter influencing siderophore mediated biocontrol activity of rhizosphere *Pseudomonas* spp. (eds. Kleister, D.L. and Cregan, P.B.. In The Rhizosphere and Plant, Kluwer Acadmeic Publishers, Netherland): pp 253-261.

Ayala, S. and Rao, E.V.S.P. (2002) Perspectives of soil fertility management with a focus on fertilizer use for crop productivity. Curr. Sci. 82: 797-807.

Bhattacharya, R. and Ray, T. (2000) Microbiological and electron microscopic studies of urea treated *Rhizobium* sp. cells. Acta. Microbiol. Polon. 49: 201-206.

Boraste, A.; Vamsi, K.K.; Jhadav, A.; Khairnar, Y.; Gupta, N.; Trivedi, S.; Patil, P.; Gupta, G.; Gupta, M.; Mujapara, A.K. and Joshi, B. (2009) Biofertilizers: A novel tool for agriculture. Int. J. Microbiol. Res. 1(2): 23-31.

Burns, R.C. and Hardy, R.W.F. (1975) Nitrogen fixation in bacteria and higher plant. Springer-Verlag, New York.

Brockwell, J. and Bottomley, P.J. (1995) Recent advances in inoculant technology and prospects for the future. Soil boil. Biochem. 27: 683-697.

Smith, R. (1992). Legume inoculant formulation and application. Can. J. Microbiol. 38: 485-492.

Smith, R.S. (1995) Inoculant formulation and application to meet changing needs. In , *nitrogen fixation fundamentals and applications.* (eds. Newton W.E., Kluwer Academic Publisher, The Netherlands): pp 653-65.

Bashan, Y. (1998) Inoculants of plant growth promoting bacteria for use in agriculture. Biotech. Adv. 16: 729- 770.

Afzal, A., and Bano, A. (2008) *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum* L.) Int. J. Agri. Biol. 10: 85-88.

Mia, M.A.B.; Shamsuddin, Z.H. and Mahmood, M. (2012) Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. Afr. J. Biotechnol. 11(16): 3758-3765.

Sanginga, N.; Ibewiro, B.; Houngnandan, P.; Vanlauwe, B.; Okogun, J. A.; Akobundu, I. O. and Versteeg, M. (1996). Evaluation of symbiotic properties and nitrogen contribution of *Mucuna* to maize grown in the derived Savanna of West Africa. Plant Soil 179: 119-129.

Bellone, C.H.; De Bellone, S.D.V.C.; Pedraza, R.O. and Monzon, M.A. (1997). Cell colonization and infection thread formation in sugar cane roots by *Azotobacter diazotrophicus*. Soil Bio. Biochem. 29: 965-967.

Postgate, J.R.; Partidge, C.D.P.; Robson, R.L.; Simpson, F.B. and Yates, M.G. (1982) A method for screening hydrogenase negative mutants of *Azotobater chroccum.* J. Gen. Microbiol. 128: 905-908.

Sprent, J.I. and Sprent, P. (1990) Nitrogen fixing organisms. Pure and applied aspects. Chapman and Hall, London, United Kingdom.

FAO (1990) Fertilizer year book FAO Rome, Italy. 79.

Dixon, R.O.D. and Wheeler, C.T. (1986). Nitrogen fixation in plants. Blackie, Glasgow, United Kingdom.

Burris, R.H. (1994). In, Nitrogen fixation with non-legumes (ed. Hegazi, N.A., Fayez, M. and Monib, M.; The American University in Cairo, Egypt). pp 1-11.

Frages, J. (1992). An industrial view of *Azospirillum* inoculant, formulation and application technology. Symbiosis 13: 15-26.

Halder, A. and Chakrabartty, P.K. (1993) Solubilization of inorganic phosphate by *Rhizobium.* Folia Microbiol. 38: 325-330.

Chabot, R.; Antoun, H. and Cescas, M.C. (1996) Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium leguminosarum* bv *phaseoli*. Plant Soil 184: 311-321.

Antoun, H.; Beauchamp, C.J.; Goussard, N., Chabot, R. and Lalande, R. (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes, effect on radishes *Raphanus sativus* L. Plant Soil 204 :57-67.

Dashi, N.; Zhang, F.; Hynes, R. and Smith, D.L. (1998) Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown *Glycine max* L. Merr. under short season conditions. Plant soil 200: 205-213.

Tien, T.M.; Gaskins, M.H. and Hubbell, D.H. (1979) Plant growth substance produced by *Azospirillum brasilense* and their effect on the growth of peral millet (*Pennisetum ammericanum* L.). Appl. Environ Microbiol. 37:1016-1024.

Hussain, A.M.; Arshad, A.M.; Hussain, A. and Hussain, F. (1987). Response of maize *Zea mays* to *Azotobacter* inoculation under fertilized and unfertilized conditions. Biol. Fertil. Soils 4**:** 73-77.

Prevost, D. ;Bordeleau, L.M.; Caaudry-reznick, S.; Schulman, H.M. and Antoun, H. (1987) Characterization of rhizobia isolated from three legumes indigenous to the Canadian high arctic, *Astragalus alpinus*, *Oxytropis maydelliana*, *Oxytropisarctobia*. Plant soil 98: 313-324.

Arora, N.K.; Kang, S.C. and Maheshwari, D.K. (2001). Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr. Sci. 81: 673-677.

Lippman, B.; Leinhos, V. and Bergmann, H. (1995) Influence of auxin producing rhizobacteria on root morphology and accumulation in maize *Zea mays* L. caused by inoculation with Indole-3-acetic acid IAA producing *Pseudomonas* and *Actinobacter* strains or IAA applied exogenously. Angew Bot. 68: 31-36.

Noel, T.C.; Sheng, C.; Yost, C.K.; Pharis, R.P. and Hynes, M.F. (1996) *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J, Microbiol. 42**:** 279-283.

Bagnasco, P.; De La Fuente, L.; Gualtieri, G.; Noya, F. and Arias, A. (1998) Fluorescent *Pseudomonas* spp. as biocontrol agents against forage legume root pathogenic fungi. Soil Biol. Biochem. 30: 1317-1322.

Beauchamp, C.J., Dion, P.; Kloepper, J.W. and Antoun, H. (1991) Phytological characteristic of opine utilizing rhizobacteria for traits related to plant growth activity. Plant Soil 132: 273-279.

Alvarez, M.A.B., Gagne, S. and Antoun, H. (1995) Effect of compost on rhizosphere microflora of the potato and on the incidence of plant growth promoting rhizobacteria. Appl Environ Microbiol 61: 194-199.

Nautiyal, C.S. (1997) Rhizosphere competence of *Pseudomonas* sp. NBRI9926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chick pea *Cicer arietinum* L. pathogenic fungi. FEMS Microbiol. Ecol. 23 : 145-158.

Chakraborty, U. and Parkayastha, R.P. (1984) Role of rhizobia toxine in protecting soybean roots from *Macrophomina phaseiolina*. Can J Microbiol. 30: 285-289.

Devine, T.E.; Kuykenendall, L.D. and O'Neill, J.J. (1988) DNA homology group and the identity of *Bradyrhizobium* strains producing rhizobitoxine-induced foliar chlorosis on soybean. Crop. Sci. 28: 939-941.

Hunter, W.J. (1993) Ethylene production by root nodules and effect of ethylene on nodulation in *Glycine max*. Appl. Environ. Microbiol. 39: 1947-1950.

Xie, Z.P.; Staehelin, C.; Wiemkemn, A. and Bolle, T. (1996) Ethylene responsiveness of soybean cultivars characterized by leaf senescence, chitinase induction and nodulation. J. Plant Physiol. 149: 690-694.

Schmidt, W.; Martin, P.; Omang, S.H. and Bangerth, M. (1999) Influence of *Azospirrilium* on nodulation of legumes. In, *Azospirrilum genetics, Physiology, Ecology*. Vol. 4. (ed by G. Klingmiller, Springlar Velrag. Heidelberg, Germany). pp 92-100.

Duodu, S.; Bhuvaneshwari, T.V.; Stokkermans, T.J. and Peters, N.K. (1999) A positive role for rhizobitoxine in *Rhizobium*-legume symbiosis. Mol. Plant Micro. Interact. 12: 1082-1089.

Carson, K.C.; Meyer, J.M. and Dilworth, M.J. (2000) Hydroxamate siderophore of root nodule bacteria. Soil Biol. Biochem. 32: 11-21.

Smith, M.J.; Sholery, J.N.; Schwyn, B.; Holden, I. and Neilands, J.B. (1985) Rhizbactin, a structurally noval siderophore from *Rhizobium meliloti*. J. Am. Chem. Soc. 107: 1739-1743.

Modi, M.; Shah, K.S. and Modi, V.V. (1985) Isolation and Characterization of catechol type siderophore from cowpea *Rhizobium* RA-1.Arch Microbiol. 141: 156-158.

Rioux, C.R.; Jordan, D.C. and Rattray, J.B.M. (1986) Iron requirement of Rhizobium leguminosarum and secretion of anthranilic acid during growth on an iron deficient medium. Arch. Biochem. Biophys. 248: 175-182.

Nambiar, P.T.C. and Sivaramkrishnan, S. (1987) Detection and assay of siderophores of cowpea rhizobia *Bradyrhizobium* using radioactive Fe (^{59}Fe). Lett. Appl. Microbiol. 4**:** 37-40.

Patel, H.N.; Chakraborty, R.N. and Desai, S.B. (1988) Isolation and partial characterization of phenolate siderophore from *Rhizobium leguminosarum* IARI 102. FEMS Microbiol Lett. 56: 131-134.

Skorupska, A.; Derylo, M. and Lorkiewicz, Z. (1989) Siderophore production and utilization by *Rhizobium trifolii*. Biol. Metals. 2: 45-49.

Guerinot, M.L.; Meidl, E.J. and Plessner, O. (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. J. Bacteriol. 172: 3298-3303.

Persmark, M.; Pittman, P.; Buyer, J.S.; Schwyn, B.; Gill, P.R.; Neilands, J.B. (1993) Isolation and structure of rhizobactin 11021, a siderophore from alfalfa symbiont *Rhizobium meliloti* 1021. J. Am. Chem. Soc. 115: 3950-3956.

Dilworth, M.J.; Carson, K.C.; Giles, R.G.F.; Byrne, L.T. and Glenn, A.R. (1998) *Rhizobium leguminosarum* bv *viciae* produces a novel cyclic trihydroxamte siderophore, vicibactin. Microbiol. 1444: 781-791.

Hanshem, F.M.; Saleh, S.A.; Van Berkem, P. and Voll, M. (1997) Survival of Bradyrhizobium sp. Arachis on fungicide-treated peanut seed in relationship to plant growth and yield. W. J. Microbiol. Biotech. 13: 335-340.

Aliwi, N.; Wynne, J.C.; Rawlings, J.O.; Schneewes, T.J. and Elkan, G.M. (1989) Symbiotic relationship between *Bradyrhizobium* strains and peanut. Crop Sci. 29: 50-54.

Taber, R.A.; Smith, O.D.; Smith, D.M.; Segner, S.L.; Taber, W.R.; Pettit, R.E.; Neck, J.S.; Rajapakse, S. and Harman, W.L. (1994). Growth response of peanut to field inoculation with endomycorrhizal fungi, *Bradyrhizobium* and supplemental phosphate in Texas. Peanut Sci. 21: 104-112.

Rebafka, F.P.; Ndungunu, B.J. and Marschner, H. (1993) Crop residue application increase nitrogen fixation and dry matter production in groundnut *Arachis hypogaea* L. grown on acid sandy soil in Niger, West Africa. Plant soil. 150: 213-222.

Cook, R.J.; Thomasshow, L.S.; Weller, D.M.; Fujimotto, D.; Mazzola, M.; Bangera, G. and Kim, D. (1995) Molecular mechanism of defence by rhizobacteria against root disease. Nat. Acad. Sci. 92: 4197-4201.

Enteshamul, H.S. and Graffar, A. (1993) Use of rhizobia in the control of root rot disease of sunflower, okra, soybean and mungbean. J. Phytopathol. 138(2): 157-163.

Paulitz, T.C. and Fernand, W.G.D. (1996) Biological seed treatments for the control of soil borne plant pathogen (eds Management of Soil Borne Diseases, Kalyani Publishers, India.) pp, 185-217.

Chao, W.L. (1990) Antagonistic activity of *Rhizobium* spp. against beneficial and plant pathogens fungi. Lett. Appl. Microbiol. 10: 213-215.

Kloepper, J.W. and Beauchamp, C.J. (1992) A review of issue related to measuring colonization of plant roots of bacteria. Can J. Microbiol. 38: 1219-1232.

Lumsden, R.D.; Lewis, J.A. and Fravel, D.R. (1995) Formulation and delivery of biocontrol agent for use against soilborne plant pathogen. In Biorational Pest Control Agents (eds Hall FR and Berry JW, Am. Chem. Soc.) pp 166-182.

Buonasissi, A.J.; Copman, R.J.; Pepin, H.F. and Eaton, G.W. (1986) Effect of *Rhizobium* spp. on *Fusarium solani* f. sp. *phaseoli*. Can. J. Plant Pathol. 8: 140-146.

Balasundaram, V.R. and Sarbhoy, A.K. (1988) Inhibition of plant pathogenic fungi by *Rhizobium japonicum*. Ind. Phytopath. 41: 128-130.

Tu, J.C. (1978) production of soybean from severe phytophthora root rot by rhizobium by *Rhizobium*. Physiolo. Plant. Patho. 12: 233-240.

Malajczuk, N.; Pearse, M. and Litchfield, R.T. (1984) Interaction between *Phytophthora cinnamomi* and *Rhizobium* isolates. Trans. Br. Mycol. Soc. 82: 491-500.

Perdomo, F.; Badel, E.R.; Alameda, M. and Schorder, E.C. (1995) *In vitro* evaluation of bacteria for the biological control of *Macrophomina phasiolina*. W. J. Microbol. Biotech. 11: 183-185.

Thies, J.E., Singelton, P.W. and Bohlool, B.B. (1991) Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. Appl. Environ. Microbiol. 57: 29-37.

Ham, G.E.; Caldwell, V.B. and Johnson, H.W. (1971) Evaluation of *Rhizobium japonicum* inoculants in soils containing naturalized population of rhizobia. Agron. J. 63: 301-303.
Boonkerd, N. and Weaver, R.W. (1982) Survival of cowpea rhizobia in soil as affected by soil temperature and moisture. Appl. Environ. Microbiol. 43: 585-589.
Dughn, M.H. and Bottomley, P.J. (1984) Soil acidity and the composition of an indigenous population of *Rhizobium trifolii* in nodules of different cultivars of *Trifolium subterranneum* L. Soil Bio. Biochem. 16: 405-411.
Abaidoo, R.C.; George, T.; Bohlol, B.B. and Singleton, P.W. (1990) Influence of elevation and applied nitrogen on rhizosphere colonization and competition for nodule occupancy by different rhizobial strains on field grown soybean and common bean. Can. J. Microbiol. 36: 92-96.
El-Sheikh, E.A.E. and Wood, M. (1990) Salt effect on survival and multiplication of chick pea and soybean rhizobia. Soil Biol. Biochem.22: 343-347.
Rao, D.L.N. and Sharma, P.C. (1995) Effectiveness of rhizobial strains for chickpea under salinity stress and recovery of nodulation on desalinization. Ind. J. Exp. Biol. 33: 500-504.
Tate, R.L. (1995) Soil microbiology (symbiotic nitrogen fixation), John Wiley and Sons, Inc. NewYork, NY. pp 307-333.
Delgado, M.J.; Ligro, F. and Liuch, C. (1994) Effects of salt stress on the growth and nitrogen fixation by pea, faba bean, common beanand soybean plants. Soil Bio Chem. 26: 371-376.
Cordovilla, M.P.; Ocana, A.; Ligero, F. and Liuch, C. (1995). Salanity effects on the growth analysis and nutrient competition in four legumes- *Rhizobium* symbiosis. J. Plant Nutr. 18: 1595-1609.
Lal, B. and Khanna, S. (1995) Selection of salt-tolerant *Rhizobium* isolates II. W. J. Microbiol. Biotech. 8 : 520-526.
Peoples, M.B.; Herridge, D.F. and Ladha, J.K. (1995) Biological N_2-fixation, An efficient source of nitrogen for sustainable agriculture. Plant Soil, 174: 1214-1222.
Idrissi, M.M.E.; Aujjar, N.; Dessaux, Y.; Filali-Maltouf, A. (1996) Characterization of rhizobia isolated from carob tree *Ceratonia siliqua*. J. Appl. Biotech. 80: 165-173.
Arora, N.K.; Kumar, V. and Maheshwari, D.K. (2000) Isolation of both fast and slow growing rhizobia effectively nodulating a medicinal legume, *Mucuna pruriens*. Symbiosis. 29: 121-137.
Le Rudulier, D. and Bernard, T. (1986) Salt tolerance in *Rhizobium*, a possible role for betaines. FEMS Microbiol. Rev. 39: 67-72.
Fougere, F. and Le Rudulier, D. (1990) Uptake of glycine betaine and it,s analogues by bacteroids of *Rhizobium meliloti*. J. Microbiol. 136, 157-163.
Ghittoni, N.E. and Bueno, M.A. (1996). Change in cellular content of trehalose in four peanut rhizobia strains cultured under hypersalinity. Symbiosis. 20: 117-127.
Ghittoni, N.E. and Bueno, M.A. (1995). Peanut rhizobia under salt stress, role of trehalose accumulation in strain ATCC 514466. Can. J. Microbiol.,41: 1021-1030.
Gouffi, K.; Pica, N.; Pichereau, V. and Blanco, V. (1999) Disaccharides as a new class of non accumulated osmoprotectants for *Sinorhizobium meliloti*. Appl. Environ. Microbiol. 65: 1491-1500.
El-Sheikh, E.A.E. and Wood, M. (1995) Nodulation and N_2 fixation by soybean inoculated with salt tolerant rhizobia or salt sensitive bradyrhizobia in saline soil. Soil Biol. Biochem. 27: 657-661.
Jenkins, M.B.; Virginia, R.A. and Jarrel, W.M. (1989) Ecology of fast growing and slow growing mesquite nodulating rhizobia in Chihuahua and Sonoran desert ecosystem. Soil. Sci. Soc. Am. J. 53: 543-549.
Hunt, P.J.; Wollum, A.G. and Matheny, T.A. (1981) Effect of soil water on *Rhizobium japonicum* infection nitrogen accumulation and yield in bragg soybean. Agric. J. 73: 501-505.
Abdel-wahab, H.H. and Zahran, H.H. (1979) Salt tolerance of *Rhizobium* species in broth culture. Z. Allg. Mikrobiol. 19: 681-685.
Abdel-wahab, H.H. and Zahran, H.H. (1983). Effect of water stress on N_2 fixation and growth of *Medicago sativa* L. Acta Agron. Acad. Sci. 32: 114-118.
Guerinot, M.L.; Meidl, E.J. and Plessner, O. (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. *J. Bacteriol.*, 172: 3298-3303.
Pararjasingham, S. and Knievel, D.P. (1990) Nitrogenase activity of cowpea *Vigna unguiculata* L. Walp. during and after water stress. Can. J. Plant Sci. 70: 163-171.
Simpson, D. and Daft, M.J. (1991) Effect of *Glomus clarum* and water stress on growth and nitrogen fixation in two genotype of groundnut. Aft. Ecosyst. Environ. 35: 47-54.
Kishinevsky, B.D.; Sen, D. and Weaver, R.W. (1992) Effect of high root temperature on *Bradyrhizobium*- peanut symbiosis. Plant Soil. 143: 275-282.
Michiels, J.; Verreth, C. and Vanderleyden, J. (1994). Effect of temperature stress on bean nodulating *Rhizobium* strains. Appl. Environ. Microbiol. 60: 1206-1212.
Graham, P.H.; Ocampo, G.C.; Ruiz, L.D. and Duque, A. (1980) Survival of *Rhizobium phaseoli* in contact with chemical seed protection. Agron. J. 72: 625-627.
Cooper, J.E., Wood, M. and Bjourson, A.J. (1985). Nodulation of *Lotus pedunculatus* in iron rooting solution by fast and slow growing rhizobia. Soil Biol. Biochem. 17: 487-492.
Rossum, D.V.; Muyotche, A. and Verseveld, H.W. (1994) Siderophore production by *Bradyrhizobium* spp. Strains nodulating groundnut. Plant Soil. 169: 177-187.
Schwertmann, U. and Taylor, R.M. (1989) In Minerals in soil environments (eds. Dixon, J.B. and Weed, S.B. Soil Sci. Soc. Am., WI).

Crichton, R.R.; Ponee-Ortiz, Y.; Koch, M.H.J.; Parfait, R. and Stuhrmann, H.B. (1978) Isolation and characterization of phytoferritin from pea *Pisum sativum* and lentil *Lens esculenta*. Biochem J. 171: 349-386.
Archibald, F. (1983) *Lactobacillus plantarum*, an organism not require iron. FEMS Microbiol. Lett. 19: 29.
Linhsay, W.L. (1979) Chemical equilibria in soil. Wiley, New York.
Neilands, J.B. (1981) Microbial iron compounds. Annu. Rev. Biochem. 50: 715-731.
Neilands, J.B. 1982. Microbial envelope protein related to iron. *Annu. Rev. Microbiol.*, 36, 285-309.
Tang, C.; Robson, A.D. and Dilwarth, M.J. (1990). The role of iron in nodulation and nitrogen fixation in *Lupinus ungustifolius* L. New Phytol. 114: 173-182.
Trevors, J.T.; van Elsan, J.D.; Lee, H. and van Overbeck, L.S. (1992) Use of alginate and other carrier for encapsulation of microbial cells for use in soil. Microb. Releases. 1: 61-69.
Somasegaran, P.; Abaidao, R.C. and Kumaga, F. (1992) Host- *Bradyrhizobium* relatio9nships and nitrogen fixation in the bambara groundnut (*Voandzeia subterranean* L.). Trop. Agric. 67: 137-142.
Kremer, R.J. and Peterson, H.L. (1983) Field evaluation of selected *Rhizobium* in an improved legume inoculant. Agro. J. 75: 139-143.
Sparrow, S.D. and Ham, G.E. (1983) Survival of *Rhizobium faseoli* in six carrier materials. Agron. J. 75: 181-184.
Van Elsas, J.D. and Van Overbeek, L.S. (1993) Bacterial responses to soil stimuli. In Starvation in bacteria (ed. Kjelleberg, S., Plenum Press, New York). pp, 55-79.
Thompson, J.A. (1980) Production and quality control of legumes inocuants. In, Methods for evaluating biological nitrogen fixation (ed. Begerson F.J., John Wiley and Sons, New York) pp,489-533.
Chao, W.L. and Alexander, M. (1984) Mineral soils as carriers for *Rhizobium* inoculants. Appl. Environ. Microbiol. 47: 94-97.
Richter, E.; Ehwald, R. and Conitz, C. (1989) Immobilization of yeast cells in plant cell wall frameworks. Appl. Microbiol. Biotech. 32: 309-312.
van Veen, J.A.; van Overbeek, L.S. and van Elsas, J.D. (1997) Fate and activity of microorganism introduced into soil. Microbiol. Molecul. Bio Rev. 61: 121-135.
Kostov, O. and Lynch, J.M. (1998) Composed sawdust as a carrier for *Bradyrhizobium*, *Rhizobium* and *Azospirillum* in crop inoculation. W. J. Microbiol. Biotech. 14, 389-397.
Van der Merwe, S.P.; Strijdom, B.W. and Uys, C.J. (1974) Groundnut response to seed inoculation under extensive agricultural practices in South African soils. Phytophylactics, 6: 295-302.
Hiltbold, A.E., Thurlow, D.L. and Skipper, H.D. (1980) Evolution of commercial soybean inoculants by various techniques. Agr. J. 72: 675-681.
Hoque, M.S. (1993) *Bradyrhizobium* technology, a promising substitute for chemical nitrogen fertilizer in Bangladesh agriculture. Plant Soil, 155: 337-340.
Terouchi, N. and Syono, K. (1990) *Rhizobium* attachment and curling in asparagus, rice and oat plants. Plant cell physiol. 31: 119-127.
Werner, D. (1992) Symbiosis of plant and microbes, Chapman and Hall, London.
Staehelin, C.; Granado, J.; Wieken, A.; Mellor, R.B.; Feliz, G.; Regenass, M.; Broughton, W.J. and Boller, T. (1994) Perception of *Rhizobium* nodulation factor by tomato cells and inactivation by root chitinase. Proc. Netl. Acad. Sci. 91: 2196-2203.
Trinick, M.J. and Hadobas, P.A. (1995) Formation of nodular structures on the non-legumes *Brassica napus, B. campestris, B. juncea* and *Arabidopsis thaliana* with *Bradyrhizobium* and *Rhizobium* isolated from *Parasponia* spp. or legumes grown in tropical soils. Plant soil. 172: 207-219.
Wiehe, W. and Höflich, G. (1995) Survival of plant growth promoting rhizosphere bacteria in the different crops and migration to non-inoculated plant under field condition in northern east Germany. Microbiol Res. 150: 201-206.
Nambiar, P.T.C. (1985) Response of groundnut *Arachis hypogaea* L. to *Rhizobium* inoculation in field, problems and prospects. MIRISAT J. 1: 293-309.
Simanungkalit, R.D.M.; Indrasumunar, A.; Pratiwi, E.; Hastuti, R.D. and Roughley, R.J. (1995). Population dynamics of bradyrhizobia in soil used for upland and lowland rice/soybean cropping system in west java, Indonesia. Soil Bio. Biochem. 27,625-628.
Olufajo, O.O. and Adu, J.K. (1993) Nodulation of soybean grown under field conditions and inoculated with *Bradyrhizobium japonicum* strains. In biological nitrogen fixation and sustainability of tropical agriculture (eds Mulongoy K, Gueye M and Spencer DSC, John Wiley and Sons, NY)pp. 147-154.
Okereke, G.U.; Onochie, C.C.; Onukwo, A.U.; Onyeagba, E. and Ekejindu, G.O. (2000) Response of introduced *Bradyrhizobium* strains infection a promiscuous soybean cultivar. W.J. Microbiol. Biotech. 16: 43-48.
Bienfait, H.F. (1989). Prevention of stress in iron metabolism of plant. Acta. Bot. Neerl., 38: 105-129.
Sugiura, Y.; Tannaka, H.; Mino, Y.; Ishida, T.; Ota, N.; Inoue, M.; Non, K.; Yoshioka, H. and Takemoto, T. (1981) Structure properties transport mechanism of iron III complex of mugineic acid, a phytosiderophore. J. Am. Chem. Soc. 103: 6979-6982.
Carson, K.C.; Holliday, S.; Glenn, A.R. and Dilwarth, M.J. (1992). Siderophore and organism acid production in root nodule bacteria. Arch. Microbiol. 157: 264-271.
Molla, A.H.; Shamsuddin, Z.H.; Halimi, M.S.; Morziah, M. and Puteh, A.B. (2001) Potential for enhancement of root growth and nodulation of soybean coinoculation with *Azospirillum* and *Bradyrhizobium* in laboratory systems. Soil Bio Biochem. 33; 457-463.
Sarig, S.; Kapulnik, Y. and Okon, Y. (1986) Effect of *Azospirillum* inoculation on nitrogen fixation and growth of several winter legumes. Plant Soil. 90: 335-342.

Del Gallo, M. and Fabri, P. (1991) Effect of soil organic matter on chickpea inoculate with *Azospirillum brasilense* and *Rhizobium leguminosarum* bv. *ciceri*. Plant and soil. 137: 171-175.

Burdman, S.; Kigel, J. and Oken, Y. (1997). Effect of *Azospirillum brasilense* on nodulation and growth of common bean *Phaseolus vulgaris* L. Soil Biol. Biochem. 29: 923-929.

Rice, W.A.; Olsen, P.E. and Leggett, M.E. (1994) Co-culture of *Rhizobium meliloti* and a phosphorous solublizing fungus *Penicillium bilaii* in sterile peat. Soil Biol. Biochem. 27: 703-705.

Anderson, K.J. (1957) The effect of soil micro-organisms on the plant-rhizobia association. Rev. Int. Bot. Exp. 8: 59-73.

Plazinski, J. and Rolfe, B.G. (1985) Influence of *Azospirillum* strains on the nodulation of clovers by *Rhizobium* strains. Appl. Environ. Microbiol. 49: 984-989.

Li, D. M. and Alexander, M. (1988) Co-inoculation with antibiotic producing bacteria to increase colonization and nodulation by rhizobia. Plant Soil. 108: 211-219.