International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 6 (3): 235-242, 2010
ISSN 1811-9700

Nitrogen Fixation and Transportation by Rhizobacteria: A Scenario of Rice and Banana

M.A. Baset Mia and Z.H. Shamsuddin
Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor DE, Malaysia

Abstract: Rhizobacteria can fix N_2 in association with rice roots externally and internally and this fixed N_2 is being utilized by the host plant. Nitrogen fixation and plant growth promotion by rhizobacteria might be important factors in achieving a sustainable rice production in the future. Attempt has been made to review recent research findings related to N_2 fixation in rice by associative rhizobacteria and summarized thoroughly for a new research arena. The findings revealed that rhizobacteria are potential inocula for N_2 fixation and utilization in rice and other non-legumineous like banana. The fixed N_2 is converted to NH_4^+ ion in the cytoplasm of bacteria and excreted to the host cytoplasm through down hill process. This release can be inhibited by the presence of ambient NH_4^+. Most of the rhizospheric fixed N_2 are being utilized after mineralization of bacterial dead body. Future research should not ignore the potential of improving rice production through endophytic rhizobial inoculation via mechanisms that involve Biological Nitrogen Fixation (BNF) process.

Key words: N_2-fixation, quantification, assimilates, colonization, rhizobacteria, rice, translocation

INTRODUCTION

Nitrogen is one of the most important nutrient inputs limiting rice production as it suffer from a mismatch in plant N demand and fertilizer-N supply, which often results in a 50-70% loss to the environment (Ladha *et al*., 1997). One of the important approaches to solve the problems is to increase the ability of the rice plant to utilize N from a continuous supply. This might be possible from fixed N_2 by rice plants through BNF process. This approach is very important for high productivity of rice especially for modern rice varieties where N-demand is very high. Biofertilizer, an alternative source of chemical fertilizer is microbial inocula can ensure a plenty environmental benefits as well as helps resource-poor farmers for rice cultivation. Inoculation of associative and free-living N_2-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed Plant Growth Promoting Rhizobacteria (PGPR) also as bioenhancer and biofertilizer (Kloepper *et al*., 1980; Bashan and Holguin, 1998; Shamsuddin *et al*., 1999). Significant increases in crop yields following application of PGPR have been recorded under diversified controlled and field conditions (Bashan, 1998; Mia *et al*., 2005). They have been widely reported to fix atmospheric N_2 with grasses, cereals and bananas (Dobereiner, 1997; Mia *et al*., 2007) and enhance nutrient uptake and drought resistance (Arzanesh *et al*., 2009; Kapulnik, 1991; Bashan and Holguin, 1997; Sheng, 2005; Mia *et al*., 2009). However, their success is much vulnerable to environmental and edaphic factors as compared to the *Rhizobium*-legume symbiotic association. Of the various naturally occurring N_2-fixing plant-microbe associations, the symbiosis between rhizobia and legume plants is the best understood scientifically. Moreover, this association is also the most successful in agriculture system which, providing fixed N_2 for many of the world's major legume crop species.

Rhizobium-nonlegume interactions in recent time have created an interesting avenue for exploring new opportunity for fixing nitrogen in rice. This approach has also been created an opportunity for manipulating of N_2-fixing gene into rice system with rice-rhizobia symbiosis system. Recent advances in accepting symbiotic at the molecular level and the ability to manipulate genes in the rice for fixing N_2 have created tremendous opportunities. It is found that the benefits of this association which leads to greater production of vegetative and reproductive biomass was more likely due to rhizobial modulation of the plant root architecture, enhanced root hair for absorbing plant nutrients efficiently rather than BNF per se (Mia and Shamsuddin, 2009; Sheng and Huang, 2001; Yanni *et al*., 2001). A significant increase in biomass and grain yield was also

Corresponding Author: Z.H. Shamsuddin, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor DE, Malaysia

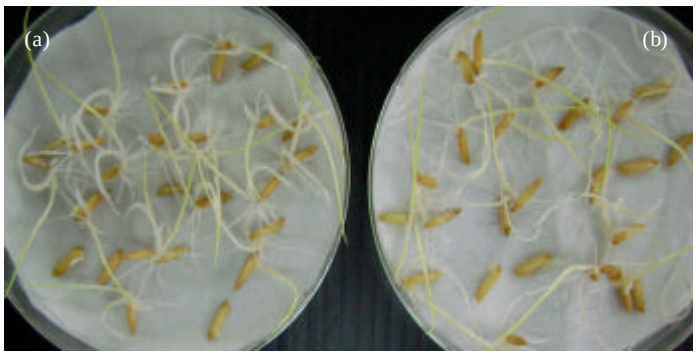

Fig. 1: Formation of more root hair of rice seedlings due to inoculation of *Rhizobium* sp. (a) inoculated with *Rhizobium* and (b) un-inoculated cintrol

Table 1: Initiation of root hair in lowland rice variety MR219 as influenced by rhizobacterial inoculation

Treatments	Initiation of root hair (hour after inoculation)	Initiation of lateral roots (hour after inoculation)	Occurrence of root hair after 96 h of inoculation
Control	72	96	-
UPMR29 *(Rhizobium* sp.)	72	96	++
UPMR48*(Rhizobium* sp.	48	72	++
SB13 (*Borkholderia* sp.)	72	72	+
SB35 (*Corynebacterium* sp.)	48	96	-
SB41 (*Corynebacterium* sp.)	48	72	-

+: Presence of root hairs, -: Absence of root hairs. Source: Mia and Shamsuddin (2009)

recorded in greenhouse-grown rice plants inoculated with these isolates (Singh *et al*., 2006; Mia and Shamsuddin, 2010). The previous studies of PGPR inoculation in bananas also showed similar beneficial effects namely growth nutrient uptake, yield and fruit quality (Mia *et al*., 2000, 2005). An experiment was conducted under laboratory condition and the results showed that *Rhizobium* inoculation enhanced the seed germination capacity, seedling vigor in lowland rice. It was also observed that inoculation significantly initiated and persisted more root hairs in germinated rice seeds (Fig. 1a, b, Table 1) (Mia and Shamsuddin, 2009).

This type of new information has stimulated rice scientists on the current research topic and encouraged to isolate rhizobial strains which are better adapted for rice colonization. Recent research has identified the existence of true rhizobial endophytes of rice plants (Singh *et al*., 2006).

The bacteroids of nodule in legume-*Rhizobium* symbiosis system are the site of biochemical pathways which takes place during N_2-fixation. They are solely dependent on the host plant for their energy requirements. Besides the bioenhancing activities, N_2 fixation by associative, endophytic or rhizospheric bacteria they released the N-product to the host plant which is important for a successful biofertilizer technology in rice cultivation system. Numerous research works have been done by rhizobacterial inoculation on different aspect of research area. However, the main beneficial effects which originated from N_2 fixation transport of fixed N_2 to the host plant and this subsequent implication remains to be elucidated. Here, attempts have been taken to summarize current information on the N_2 fixation of rhizobacteria in association with rice and their transportation unlike *Rhizobium*-legume symbiosis.

Colonization of bacteria with host roots: For a positive interaction between PGPR and rice the bacteria must come into contact with the roots for a successful colonization. Without a good and secure attachment the beneficial rhizobacteria can not perform positive effects. Root colonization of beneficial microbs is always considered a major factor in successful inoculation of plants by bacteria (Suslow, 1982) which is commonly known to function in four stages: (1) primarily the movement of microbes to the plant root surface. Which either can be passive, through soil water fluxes, or active, via specific induction of flagellar activity by plant-released compounds generally termed as chemotaxis. Secondly bacterial adsorption to the root surface. Following adsorption and anchoring, specific and/or complex interaction between the bacterium and the host plant may ensue, which lead to induction of bacterial gene activity (Brimecombe *et al*., 2001). Majority of the PGPR-nonlegume association bacteria form colonies the root superficially and few cases extent internally (Gallo and Fendrik, 1994; Mia *et al*., 1999). Bacterial movement can play significant role in the survivility of bacteria in the soil and rhizosphere where attachment is also very important for beneficial effects (Turnbull *et al*., 2001).

In rice, the *Rhizobium* can be colonize externally, i.e., on the root surface and internally almost in the apoplastic region. It is difficult to invade *Rhizobium* into the cytoplasm though plsmamembrane as the membrane pore size is smaller than bacterial size. Anyway, the secure attachment of PGPR is essential for a long-term association with the host plant roots for three reasons:

- If the bacteria are not attached to root epidermal cells, bacterial released substances will diffuse into rhizosphere and might be consumed by other non-beneficial microbes before being taken up by the plants
- Without an assured attachment, bacteria may wash away from the rhizosphere
- There is a chance to colonize by other aggressive and possibly non-beneficial, colonizers if the association site is remained vacant (Bashan and Holguin, 1997)

The previous study of root colonization in bananas showed that rhizobacteria strains Sp7 and UPMB10 could successfully form colonies on the root surface of bananas. The bacteria can remain inside the intercellular space i.e., apoplastic in nature and the bacteria can not move through transmembrane (Fig. 2, 3) (Mia *et al*., 1999). This has demonstrated that bacterial colonization occurred mainly on root surface and more cells were observed in the root hair proliferation zone. The latter might be due to the presence or availability of root exudates in this area. Similarly, inoculation with *Herbaspirillum* sp., *Burkholderia* sp. and *A. brasilense* has been shown to colonize roots of maize, rice and wheat, respectively (Dobereiner, 1997). Puente *et al.* (1999) found massive colonization of *A. brasilense* and *A. halopraeferens* on root surface of mangrove seedlings grown in sea water which is very important for growth and development of the host plant.

Recently, endophytic N_2-fixing bacteria have been gaining prominence where diazotrophic bacteria colonize roots, stems and even leave of some cereals internally. The advantages of this system are those where they probably undergo less competition with other microorganisms for carbon substrates than rhizosphere bacteria. Possibly they can excrete part of their fixed N_2 directly into the host plants (Baldani *et al*., 2000). Higher and even the lower plants have endophytes that can be found intercellularly, *Cyanobacterium flavescens* and *Bacillus pumilus* could colonize intercellularly in rice seedlings where they contribute the growth and development of host plants (Bacilico-Jimenz *et al*., 2001).

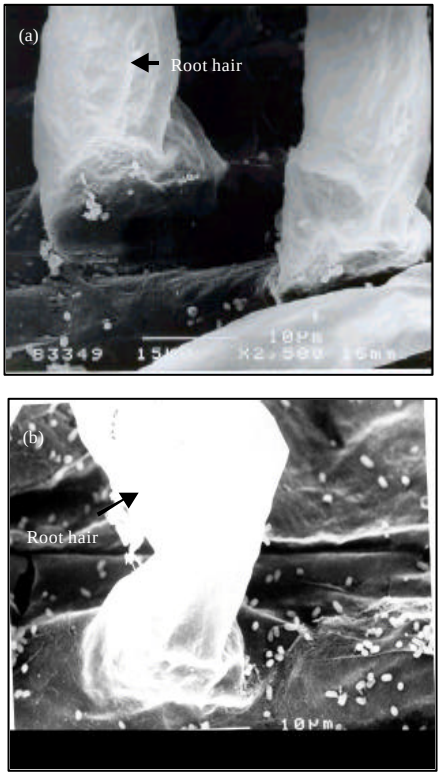

Fig. 2: SEM micrographs of inoculated banana root colonization by PGPR, root surface showing bacterial cells at root hair proliferation zone: (a) Sp7 and (b) UPMB10; arrow showing root hair devoid of bacterial cells; bar represents 10 µm

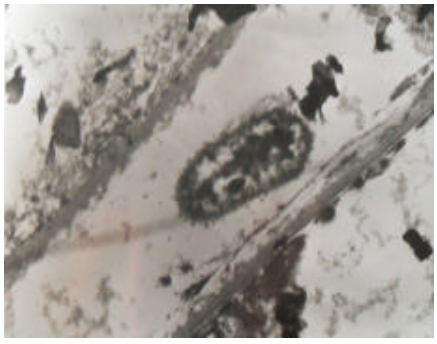

Fig. 3: Transmission Electron Micrograph (TEM) of inoculated banana root colonization by rhizobacteria intercellular space showed presence of bacteria

Rhizobium and other PGPR are known as effective colonizers of cereals (Baldani *et al*., 1986), vegetables (Hadas and Okon, 1987) and mangrove plants (Puente *et al*., 1999). However, root colonization pattern

vary greatly among PGPR strains (Bashan and Levanony, 1990). A higher frequency of bacteria are observed by many scientists in the middle lamellae, an area of longitudinal contact between epidermal cells of the roots although root tips of many plants are not colonized by bacteria (Bowen and Rovira, 1976; Foster and Bowen, 1982).

Fixation of N_2 in association with rice roots: Among the mechanisms of improved plant growth in rhizobacteria inoculated plants BNF was the first and most pronounced effect. This is due to increased N-compound in the inoculated plants and the activity of nitrogenase enzyme (Bashan and Holguin, 1997; Mia *et al*., 2010a). Findings of experimental results based on different techniques namely N-balance, ^{15}N isotope dilution and ^{15}N natural abundance have provided strong evidence that some tropical grasses, especially sugar cane, lowland rice and kallar grass (*Leptochloa fusca*), oil palm and banana can get at least part of their N-needs from BNF process (Amir *et al*., 2005; Mia *et al*., 2007; James, 2000). The BNF is limited to prokaryotic bacteria and a genomic survey reported approximately 5% of prokaryotes carry N_2 fixing genes (Raymond *et al*., 2004). When atmospheric N_2 is converted to ammonia through biological process then it is called Biological Nitrogen Fixation (BNF), where two moles of ammonia are produced from one mole of N_2. The enzyme nitrogenase play dominant role in the conversion where the diazotrophs contained the enzyme complex for the reduction process. The enzyme protein of nitrogenases is a complex of dinitrogenase (MoFe protein) and dinitrogenase reductase (Fe protein) (Burris, 1991).

Quantification of BNF: Several methods have been developed for measuring BNF; some of them are only qualitative and not very useful for quantification purposes (Danso, 1985). The most appropriate methods are those that can distinguish between the proportions of plant N derived from atmospheric N_2 fixation, distinct from the N contribution from soil and where relevant, from fertilizer applied (Danso, 1995). Several established methods are used for BNF quantification. The Total Nitrogen Difference (TDN) is the oldest and most traditional method for estimating the N_2-fixation rate. The increase of total N as measured by Kjeldahl method is the indication of fixed N_2. However, this has some problems, is not accurate and cannot be used in fertile soil.

The Acetylene Reduction Assay (ARA) technique indirectly measures BNF by estimating nitogenase enzyme activity. This involves short-term analyses and not easy to conduct under field conditions. Several scientists have found the disadvantages of this process and suggested that this technique is applicable for a short period under certain special conditions (Lethbridage *et al*., 1982; Witty, 1979; Danso, 1995). This technique can be useful as *in situ* measurements of nitrogenase activity at a point in time. However, this ARA technique can not estimate the actual amounts of fixed N_2 as the assay only estimates the nitrogenase activity (Boddey, 1987; Boddey *et al*., 1995).

Cell sap analysis is another technique of estimating N_2-fixation where analyses of N solute in xylem exudates and plant parts is based on the determination of the composition of N compounds in plant tissues or N flowing through the xylem sap to the shoot. However, the method is severely challenged by the fact that only a small proportion of known N_2 fixing plants are ureide exporters.

The $^{15}N_2$ was used to provide direct method for detecting BNF soon after ^{15}N became available although has limited practical or field applications in terms of quantifying BNF (Danso, 1995). The ^{15}N can be used both in isotopic enrichment and dilution techniques where the dilution technique was first described and applied to measure the contribution of BNF to clover grown in pots by McAulife *et al*. (1958). This method depends upon differences in isotopic composition of the sources of N available for plant growth, i.e., soil N, fertilizer N and atmospheric N_2 (Bergersen and Turner, 1983). This technique has the potential of being able to separately measure any plant-associated contribution of BNF to plants. This is more versatile and adoptable to various experimental conditions (Boddey *et al*., 1996; Roger and Ladha, 1992). It has been widely used to estimate the contribution of N_2 fixation to the legumes (Rennie *et al*., 1978; Malik *et al*., 1988) and also to cereals inoculated with rhizobacteria in the field (Rennie, 1980). This method has been applied for the measurement of associative N_2 fixation in non-leguminous crops viz., bananas, kallar grasses (Mia *et al*., 2007; Malik *et al*., 1997). The previous studies of the estimation of fixed N_2 of rhizobacteria inoculated tissue-cultured bananas were done by ^{15}N isotopic dilution technique (Table 2). The inherent simplicity of this method makes it the method of choice for common purpose. However, non-fixing reference plant is the ultimate source of error in this methodology, when the temporal and spatial distribution of the isotope is non-uniform (Chalk and Ladha, 1999).

Distribution of N-compounds: The release of ammonium by N_2-fixing rhizobacteria linked with economically important corps is of great interest. This is a raising question whether the fixed N_2 can be utilized by the host plant easily or not. In *Rhizobium*-legume association, the fixed-N_2 is released to host cytoplasm where they convert

Table 2: Distribution of % ^{15}N atom excess in root, pseudostem and leaves of PGPR inoculated plants at various levels of fertilizer-N

		Atom excess (%)			
		Fertilizer N-levels (ppm)			
	Treatments	3.2	8	20	50
Root	Control	5.85a	7.55a	8.48a	9.02a
	Sp7	3.71b	5.81b	7.38b	8.49b
	UPMB10	3.63b	6.08b	7.55b	8.45b
Pseudostem	Control	9.29a	9.47a	9.31a	9.52a
	Sp7	5.32b	7.02b	8.24b	8.58b
	UPMB10	4.35b	7.20b	8.01b	8.84b
Leaf	Control	6.36a	8.08a	8.75a	9.12a
	Sp7	4.10b	6.11b	7.66b	8.82b
	UPMB10	3.91b	6.29b	7.60b	8.71b

Means having same letter(s) in a column of a parameter do not differ significantly at 0.05 significant levels by DMRT

Table 3: Percentage of N derived from atmosphere (%Ndfa) in banana plantlets (total plant basis) inoculated with PGPR strains Sp7 and UPMB10 and supplied with various fertilizer-N levels grown under hydroponics condition

	Ndfa (%)			
	Fertilizer N-levels (ppm)			
Treatments	3.2	8	20	50
Control	0.0b (x)	0.0b (x)	0.0b (x)	0.0b (x)
Sp7	37.0a (w)	24.3a (x)	12.4a (y)	5.0a (z)
UPMB10	39.3a (w)	22.1a (x)	12.5a (y)	5.3a (z)

Means having same letter(s) in a column or a row (parenthesis) do not differ significantly at 0.05 level by DMRT. Source: Mia *et al.* (2007)

to different amino acids. The amino acids can be transported through xylem system to leaf mesophyll tissue or directly the ammonia can be transported to the mesophyll. Generally, glutamine is a minor exported solute of N, being again converted to asparagine in temperate species and to the ureides, allantoin and allantoic acid in tropical species (Atkins, 1987). The fixed N_2 converted to amino acids via GS-GOGAT pathway in bacteria. In rice, the enzyme activities of nitrogenase and Glutamine Synthetase (GS) of some PGPR like *Klebsiella* found higher in rhizosphere than the free-living state. Transfer of fixed-N_2 and assimilation in the rice plant is not quite easy system which depends upon a flow of associative fixing system and the problem that needs to be solved in order to improve the efficiency of associative nitrogen fixation.

In associative PGPR, various ammonium excreting mutants have been developed by selecting of ethylene diamene which can excrete ammonium in the growth medium (Machado *et al.*, 1990; Christiansen-Weniger and van Veen, 1991). It is seemed that NH_4^+ transporter is more active in the presence of excreted NH_4^+ from the diazotroph. Kleiner (1985) also proposed an active ammonium uptake mechanism to maintain the higher intracellular ammonium concentration and most of the fixed ammonium by PGPR are repressed by ammonium (Kleiner, 1985). Excretion of NH_4^+ from PGPR mutants is the results of alteration of genetic changes in enzyme system of rhizobacteria. The reason for limited supply of NH_4^+ to the host plant is due to high affinity of membrane protein for NH_4^+ which prevents the easy release of that ion (Kleiner, 1981). Both rhizobacteria and host cell have ammonia transporter which aided to overcome this barrier for NH_4^+ release (Kleiner, 1985).

However, the amount of N_2 fixed by PGPR and its transfer to their host plants vary greatly (Kapulnik *et al.*, 1985; Boddey *et al.*, 1986; Kucey, 1988). Investigations using the ^{15}N isotope dilution technique indicated that most of the N_2 fixed remained to the rhizosphere, probably bound with bacterial cells and transported very little to the shoot (Nayak *et al.*, 1986; Boddy and Dobereiner, 1995). The reason for the limited N supply from associative N_2 fixation is probably because PGPR, like other free-living diazotrophs but in contrast to symbiotically living *Rhizobia* does not release fixed N_2 to its environment from the bacterial cell. This also might be due to excretion barrier as membrane protein prevents the easy release of NH_4^+ to the environment. However, our previous study on N_2 fixation by ^{15}N isotopic dilution technique with 45 days old seedlings conclusively indicated that rhizobacterial inoculation with 33% fertilizer-N (50 ppm) could fix 8.85 to 9.69 mg N/plant (5.0-5.3% Ndfa) (Table 3). This rate of N_2 fixation was further increased (10.26-10.85 mg plant^{-1}; 12.4-12.5% Ndfa) with lesser inorganic-N supply, 13% fertilizer-N (20 ppm) due to a synergistic effect between the rhizobacteria and fertilizer-N.

Similarly, Reis *et al.* (2000) found that association of *Herbaspirillum seropedicae* and *A. brasilense* with rice seedlings grown in monoaxenic agar cultures could contribute up to 54% N from fixation process. In field condition, rice plant could obtain 20% of their total N requirement by PGPR inoculation (Shrestha and Ladha, 1996). The rhizobacteria inoculation has been shown to potentially supply approximately 25-50% of N for oil palm in nursery condition (Amir *et al.*, 2005).

James and Olivares (1998) reviewed and indicated that endophytic diazotrophs may also fix N_2 in plant and transfer the fixed-N products to their hosts. Recent evidence of significant BNF contribution in economically important graminaceous crops, particularly sugar cane (Urquiaga *et al.*, 1992), rice (Shrestha and Ladha, 1996) and forage grasses, such as kallar grass (Malik *et al.*, 1997) has generated tremendous interest on N_2 fixation by PGPR.

The beneficial effect of some PGPR strains on host plants is not solely by N_2 fixation rather due to increase in nitrate assimilation. The PGPR stimulate nitrate reduction in roots and thus decrease nitrate translocation to the

leaves. It might be said with certainty that the remarkable beneficial effects of such inoculation are mainly due to plant-associated N_2 fixation and not to other factors (Michiels *et al*., 1989; Bashan and Levanony, 1990; Sumner, 1990).

CONCLUSION

It is clear from the recent information that rhizobacteria could potentially fix N_2 and transport the fixed N_2 to the host cell. The fixed N_2 is converted to NH_4^+ in the cytoplasm of bacteria and subsequently excreted to the host cytoplasm through down hill process. But present results conclusively indicated that PGPR can contribute at least 13% of their N requirement (Mia *et al*., 2010b). However, assimilation of fixed N_2 and the subsequent translocation is not studied comprehensively. This would be more convenient for the endophytic PGPR especially *Rhizobium* where bacterial released ammonium can easily be diffused to host cytoplasm. However, it is new avenue where more research is needed on the interaction between cereal grains and rhizobia or rhizobia-like bacteria.

REFERENCES

Amir, H.G., Z.H. Shamsuddin, M.S. Halimi, M.F. Ramlan and M. Marziah, 2005. Enhancement in nutrient accumulation and growth of oil palm seedlings caused by PGPR under field nursery conditions. Commun. Soil Sci. Plant Anal., 36: 2059-2066.

Arzanesh, M.H., H.A. Alikhani, K. Khavazi, H.A. Rahimian and M. Miransari, 2009. *In vitro* growth of wheat (*Triticum aestivum* L.) seedlings, inoculated with *Azospirillum* sp. snder drought stress. Int. J. Botany, 5: 244-249.

Atkins, C.A., 1987. Metabolism and translocation of fixed nitrogen in the nodulated legume. Plant Soil, 100: 157-169.

Bacilico-Jimenz, M., S. Aguilar-Flores, M.V. del Valle, A. Perez, A. Zepeda and E. Zenteno, 2001. Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. Soil Biol. Biochem., 33: 167-172.

Baldani, V.L.D., A.M.D.E.B. Alvarez, J.I. Baldani and J. Dobereiner, 1986. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and roots of the field-grown wheat and sorghum. Plant Soil, 90: 35-46.

Baldani, V.L.D., J.I. Baldani and J. Dobereiner, 2000. Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol. Fertil. Soils, 30: 485-491.

Bashan, Y. and G. Holguin, 1997. *Azospirillum*-plant relationships: Environmental and physiological advances (1990-1996). Can. J. Microbiol., 43: 103-121.

Bashan, Y. and G. Holguin, 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol. Biochem., 30: 1225-1228.

Bashan, Y. and H. Levanony, 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can. J. Microbiol., 36: 591-608.

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

Bergersen, F.J. and G.L. Turner, 1983. An evaluation of 15N method for estimating nitrogen fixation in a subterranean clover-perennial ryegrass sward. Aust. J. Agric. Res., 34: 391-401.

Boddey, R.M., V.L.D. Baldani, J.I. Baldani and J. Döbereiner, 1986. Effect of inoculation of *Azosprillum* sp. on nitrogen accumulation by field-grown wheat. Plant Soil, 95: 109-121.

Boddey, R.M., 1987. Methods for quantification of nitrogen fixation associated with Gramineae. Crit. Rev. Plant Sci., 6: 209-266.

Boddey, R.M. and J. Dobereiner, 1995. Nitrogen-associated with grasses and cereals: Recent progress and perspective for the future. Fertil. Res., 42: 241-250.

Boddey, R.M., O.C. de Oliveira, S. Urquiaga, V.M. Reis, F.L. Olovares, V.L.D. Baldani and J. Dobereiner, 1995. Biological nitrogen fixation associated with sugar cane and rice: Contribution and prospects for improvements. Plant Soil, 174: 195-209.

Boddey, R.M., B.J.R. Alves and S. Urquiaga, 1996. Evaluation of Biological Nitrogen Fixation Associated with Non-Legumes. In: Nitrogen Fixation with Non-Legumes, Malik, K.A. *et al.* (Eds.). Kluwer Academic Publishers, London, pp: 287-306.

Bowen, D.G. and A.D. Rovira, 1976. Microbial colonization of plant roots. Annu. Rev. Phytopathol., 14: 121-144.

Brimecombe, J.M., F.A. de Leij and J.M. Lynch, 2001. The Effect of Root Exudates on Rhizosphere Microbial Population. In: The Rhizosphere, Pinton, R., Z. Varanini and P. Nannipieri (Eds.). Marcel Dekker Inc., New York, USA., pp: 95-140.

Burris, R.H., 1991. Nitrogenases. J. Biol. Chem., 266: 9339-9342.

Chalk, P.M. and J.K. Ladha, 1999. Estimation of legume symbiotic dependence: An evaluation of technique based on 15N dilution. Soil Biol. Biochem., 31: 1901-1917.

Christiansen-Weniger, C. and J.A. van Veen, 1991. NH4+-excreeting *Azospirillum brasilense* mutants enhances nitrogen supply of a wheat host. Applied Environ. Microbiol., 57: 3006-3012.

Danso, S.K.A., 1985. Methods of Estimating Biological Nitrogen Fixation. In: Biological Nitrogen Fixation in Africa, Ssali, H. and S.O. Keya (Eds.). MIRCEN, Nirobi, pp: 213-244.

Danso, S.K.A., 1995. Assessment of biological nitrogen fixation. Nutrient Cycling Agroecosyst., 42: 33-41.

Dobereiner, J., 1997. Biological nitrogen fixation in tropics: Social and economic contribution. Soil Biol. Biochem., 29: 771-774.

Foster, R.C. and G.D. Bowen, 1982. Plant Surfaces and Bacterial Growth: The Rhizosphere, Phytopathogenic Prokaryotes. Academic Press, New York, pp: 159-185.

Gallo, M. and I. Fendrik, 1994. The Rhizosphere and *Azospirillum*. In: *Azospirillum* Plant Associations, Okon, Y. (Ed.). CRC Press, Boca Raton, Florida, pp: 57-75.

Hadas, R. and Y. Okon, 1987. Effect of *A. brasilense* on root morphology and respiration in tomato seedlings. Biol. Fertil. Soils, 5: 241-247.

James, E.K. and F.L. Olivares, 1998. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. Crit. Rev. Plant Sci., 17: 77-119.

James, E.K., 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res., 65: 197-209.

Kapulnik, Y., 1991. Non-Symbiotic Nitrogen Fixing Microorganisms. In: Plant Roots: The Hidden Half, Waisel, Y., A. Eshel and U. Kafkafi (Eds.). Marcel Dekker, New York, pp: 703-716.

Kapulnik, Y., R. Gafny and Y. Okon, 1985. Effect of *Azospirillum* spp. inoculation on root development and NO3-uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponics systems. Can. J. Bot., 63: 627-631.

Kleiner, D., 1981. The transport of NH3 and NH4 through biological membranes. Biophys. Acta, 639: 41-52.

Kleiner, D., 1985. Bacterial ammonium transport. FEMS Microbiol. Rev., 32: 87-100.

Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth, 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature, 286: 885-886.

Kucey, R.M.N., 1988. Alteration of size of wheat root systems and nitrogen fixation by associative nitrogen-fixing bacteria measured under field condition. Can. J. Micribiol., 34: 735-739.

Ladha, J.K., F.J. de Brujin and K.A. Malik, 1997. Introduction assessing opportunities for nitrogen fixation in rice a frontier project. Plant Soil, 194: 1-10.

Lethbridage, G., M.S. Davidson and G.P. Sparling, 1982. Critical evaluation of the acetylene reduction test for estimating the nitrogenease activity of nitrogen fixing bacteria associated with the roots of wheat and barley. Soil Biol. Biochem, 14: 27-35.

Machado, H.B., S. Funayama, L.U. Rigo and F.O. Pedrosa, 1990. Excretion of ammonium by *Azospirillum brasilense* mutants resistant to ethylenediamine. Can J. Microbial., 57: 549-553.

Malik, K.A., B. Rakhshanda, F. Azam and M.I. Sajjad, 1988. Quantification of N2-fixation and survival of inoculated diazotrophs associated with kallar grass. Plant Soil, 108: 43-51.

Malik, K.A., B. Rakhshanda, S. Mehnaz, G. Rasul, M.S. Mirza and S. Ali, 1997. Association of nitrogen-fixing plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil, 194: 37-44.

McAulife, C., D.S. Chamblee, H. Uribe-Arango, W.W. Jr. Woodhouse, 1958. Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by ^{15}N. Agron. J., 50: 334-337.

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 1999. External and internal root colonization of *Azospirillum brasilense* on tissue-cultured banana plantlets. Proceedings of 8th Scientific Conference Electron Microscopy Society, (SCEMS'99), Genting Highlands, Pahang, Malaysia, pp: 173-174.

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2000. Growth and physiological attributes of hydroponically-grown bananas inoculated with plant growth promoting rhizobacteria. Trans. Malaysian Soc. Plant Physiol., 9: 324-327.

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2005. High- yielding and quality banana production through plant growth promoting rhizobacterial inoculation. Fruits, 60: 179-185.

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2007. Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa, 16: 11-15.

Mia, M.A.B. and Z.H. Shamsuddin, 2009. Enhanced emergence and vigour seedling production of rice through plant growth promoting bacterial inoculation. Res. J. Seed Sci., 2: 96-104.

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2009. The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. Afr. J. Biotechnol., 8: 5855-5866.

Mia, M.A.B. and Z.H. Shamsuddin, 2010. *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. Afr. J. Biotechnol.,

Mia, M.A.B., Z.H. Shamsuddin and M. Mahmood, 2010a. Use of plant growth promoting bacteria in banana: A new insight for sustainable banana production. Int. J. Agric. Biol.,

Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2010b. Rhizobacterial inoculation on growth and nitrogen incorporation in tissue-cultured *Musa* plantlets under nitrogen-free hydroponics condition. Aust. J. Crop Sci., 4: 85-90.

Michiels, K., J. Vanderleyden and A. Van Gool, 1989. *Azospirillum* plant root association: A review. Biol. Fert. Soil, 8: 356-368.

Nayak, D.N., J.K. Ladha and I. Watanabe, 1986. The effect of marker *Azospirillum lipoferum* inoculated into rice its effect on growth, yield and N2 fixation of plants studied by acetylene reduction, 15N2 feeding and 15N-dilution studies. Biol. Fertil. Soil, 2: 7-14.

Puente, M.E., G. Holguin, R.G. Bernard and Y. Bashan, 1999. Root-surface colonization of black mangrove seedlings by *Azospirillum halopraeferens* and *Azospirillum brasilense* in sea water. FEMS Microbiol. Ecol., 29: 283-292.

Raymond, J., J.L. Siefert, C.R. Staples and R.E. Blankenship, 2004. The natural history of nitrogen fixation. Mol. Biol. Evol., 21: 541-554.

Reis, V.M., J.I. Baldani, V.L.D Baldani and J. Dobereiner, 2000. Biological N2 fixation in graminae and palm trees. Crit. Rev. Plant Sci., 19: 227-247.

Rennie, R.J., 1980. 15N-isotope dilution as a measure of dinitrogen fixation by *Azospirillum brasilense* associated with maize. Can. J. Bot., 58: 21-24.

Rennie, R.J., D.A. Rennie and M. Fried, 1978. Concept of 15N Usage in Dinitrogen Fixation Studies. IAEA, Vienna, pp: 107-133.

Roger, P.A. and J.K. Ladha, 1992. Biological N2 fixation in wetland rice fields: Estimation and contribution to nitrogen balance. Plant Soil, 141: 41-55.

Shamsuddin, Z.H., A.H. Ghazali and M.A.B. Mia, 1999. *Azospirillum* as a bioenhancer and biofertilizer for banana and oil palm seedlings. Biotechnol. Sustain. Util. Biol. Resour. Trop., 13: 326-338.

Sheng, X.F. and W.Y. Huang, 2001. Physiological characteristics of strain NBT of silicate bacterium. Acta Pedologica Senica, 38: 569-574.

Sheng, X.F., 2005. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol. Biochem., 37: 1918-1922.

Shrestha, R.K. and J.K. Ladha, 1996. Genotypic variation in promotion of rice nitrogen fixation as determined by nitrogen 15N dilution. Soil Sci. Soc. Am. J., 60: 1815-1821.

Singh, R.K., P.N.M. Ravi, K.J. Hemant, K.S.P.P. Vinod, B.R. Sasi and A. Kannepalli, 2006. Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. Curr. Microbiol., 52: 345-349.

Sumner, M.E., 1990. Crop response to *Azospirillum* inoculation. Adv. Soil Sci., 12: 53-123.

Suslow, T.V., 1982. Role of Root-Colonizing Bacteria in Plant Growth. In: Phytopathogenic Prokaryotes, Mount, M.S. and G.H. Lacy (Eds.). Vol. 1. Academic Press, New York, pp: 186-223.

Turnbull, G.A., J.A.W. Morgan, J.M. Whipps and J.R. Saunders, 2001. The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment of colonization of wheat roots. FEMS Microbiol. Ecol., 36: 21-31.

Urquiaga, S., K.H.S. Cruz and R.M. Boddey, 1992. Contribution of nitrogen fixation to sugarcane: ^{15}N and nitrogen balance estimates. Soil Sci. Soc. Am. J., 56: 105-114.

Witty, J.F., 1979. Acetylene reduction assay can over estimate nitrogen fixation in soil. Soil Biol. Biochem., 11: 209-210.

Yanni, Y.G., R.Y. Rizk, F.K.A. El-Fattah, A. Squartini and V. Corich *et al*., 2001. The beneficial plant-growth promoting association of *Rhizobium leguminosarum* bv. Trifolii with rice roots. Aust. J. Plant Physiol., 28: 845-870.