



Review Article

Use of Plant Growth Promoting Bacteria in Banana: A New Insight for Sustainable Banana Production

M.A. BASET MIA[†], Z.H. SHAMSUDDIN¹ AND MAZIAH MAHMOOD[‡]

Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor DE, Malaysia

[†]*Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh*

[‡]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor DE, Malaysia*

¹Corresponding author's e-mail: zulsham@agri.upm.edu.my; miabaset@yahoo.com

ABSTRACT

Banana, an important fruit crop, requires high amounts of chemical fertilizers for commercial cultivation, which is costly and can be hazardous to the environment, when used excessively. Plant growth promoting rhizobacteria (PGPR) could be used for growth promotion, nutrient uptake and some time as an alternative source of N-fertilizer of non-leguminous crops. Recently, research on PGPR for crop improvements are gaining prominence and thousands of research works have been published so far. However, use of this noble technique in banana production system is limited. Nevertheless, reports from various experimental findings suggested that PGPR strains could successfully formed colonies on the root surface of bananas, where more bacterial cells were found in the root hair proliferation zone. Application of PGPR alone could not produce significant benefits that require minimal or reduced levels of fertilizer-N consequently could produce a synergistic effect on root growth and development. The inoculation also increased the N yield and fixed N₂ in association with banana roots subsequently increased the yield, improved the physical attributes of fruit quality and initiated early flowering. The summarized review suggested that PGPR are effective as a bioenhancer and biofertilizer for banana cultivation. For consistent and precise results extensive field experiments of bananas inoculated with PGPR strains should be continued. © 2010 Friends Science Publishers

Key Words: PGPR; Banana; Growth; Nitrogen fixation; Fruit yield

INTRODUCTION

Banana is always referred to as a gross feeder and requires large amounts of nitrogen and potassium followed by phosphorus, calcium and magnesium to maintain high yields (Robinson, 1996; Abdullah *et al.*, 1999). The physiological limitation in N-storage capacity is also a constraint for commercial cultivation of this crop, the deficiency symptoms quickly develop and extra N must be frequently applied even on fertile soil (Robinson, 1996). To fulfill the plant demand for nutritional attributes it is essential to apply those elements in the soil, which mostly comes from inorganic chemical sources. The increased use of chemical fertilizer is undesirable, however because (1) its production is an energetically costly process and most of the energy is provided by the consumption of non-renewable fossil fuels and (2) considerable pollution is caused through both the production and use of mineral N-fertilizers and this is exacerbated by the relatively low efficiency of their uptake by the plants due to nonextensive root system (Ladha & Reddy, 1995; Ladha *et al.*, 1997).

Banana roots are adventitious and proliferate horizontally in the topsoil and they can not acquire nutrients

and water from the deeper soil profile like other fruit crops. This undeveloped root system limits the large-scale production of bananas under adverse tropical soil conditions, where the root systems are crucial for plant support, nutrient and water acquisition and production of plant growth regulators by rhizobacteria (de Langhe *et al.*, 1983; Stover & Simmonds, 1987; Kapulnik, 1991; Price, 1995).

Biofertilizers, microbial inoculants that can promote plant growth and productivity is internationally accepted as an alternative source of N-fertilizer. It is environmental friendly and can be used to ensure a sustainable banana production. In this biofertilizer technology new systems are being developed to increase the biological N₂ fixation (BNF) with cereals and other non-legumes by establishing N₂-fixing bacteria within the roots (Cocking, 2000). Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective biofertilizer. Inoculation of associative and free-living N₂-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1980; Bashan & Holguin, 1998). Significant increases in

crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998). They have been widely reported to fix atmospheric nitrogen with grasses and cereals (Dobereiner, 1997) and enhance nutrient uptake (Lin *et al.*, 1983; Murty & Ladha, 1988; Sarig *et al.*, 1988; Kapulnik, 1991; Bashan & Holguin, 1997). Responses of PGPR to different fruit crops are not quite similar to those found in gramineous crops.

Recently, PGPR strain UPMB 10 (*Bacillus sphaericus*), isolated from oil palm, observed to produce beneficial effects on plantation crops namely oil palm (Amir, 2001), coconut and banana (Shamsuddin *et al.*, 2000; Mia *et al.*, 2005, 2007 & 2009).

In the past 40 years, greenhouse and field inoculation studies with PGPR have shown that these rhizobacteria are able to promote yield of agriculturally important crops grown under different soil and climatic conditions (Okon & Labandera-Gonzales, 1994). However, information gathered on the inoculation of PGPR on bananas biofertilizer and bioenhancer activities have been summarized here and explored the possibilities for commercial cultivation.

Enhanced root growth by PGPR inoculation: The interest in soil microorganisms has rapidly increased, because of a greater realization of their importance in the maintenance of soil fertility. Some of the associative and free-living rhizosphere bacteria exert beneficial effects and enhance growth of many crop plants; hence they are called plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1980; Bashan & Holguin, 1998). Plant growth may be stimulated by bacterially produced phytohormones (Tien *et al.*, 1979; Okon & Labandra-Gonzalez, 1994; Yanni *et al.*, 2001), vitamin-related products (Phillips *et al.*, 1999) or pathogen suppression activities (Haque & Gaffer, 1993; Bashan & de Bashan, 2002). The most likely candidate for PGPR is *Acetobacter diazotrophicus*, *Herbaspirillum* spp. for sugar cane (Boddy *et al.*, 1995; Dobereiner, 1995; Baldani, *et al.*, 1997), *Azoarcus* spp. in kallar grass (Reinhold & Hurek, 1998) and species of *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Burkholderia*, *Cellulomonas*, *Frankia*, *Pantoea*, *Pseudomonas*, *Serratia*, *Streptomyces* and *Thiobacillus* for different legumes and non-legumes (Patriquin *et al.*, 1983; Boddey *et al.*, 1995; Triplett, 1996; Malik *et al.*, 1997; Stoltzfus *et al.*, 1997; Yanni *et al.*, 1997; James, 1999). Mia (2002) found enhanced root formation in bananas, when inoculated with PGPR strain Sp 7 and UPMB10 (Plate 1). The plant growth promoting effects of PGPR are mainly derived from morphological and physiological changes in roots of inoculated plant (Okon *et al.*, 1988; Sarig *et al.*, 1988). The inoculation process could stimulate the root growth and development (Mia *et al.*, 1998), which occurred almost in all dimensions namely production of primary and secondary roots, longer roots and greater volume and mass (43%).

Colonization of PGPR on roots of crop plants: Root

colonization is always considered a major factor in successful inoculation of plants by beneficial bacteria (Suslow, 1982), which is known to involve four stages. The initial stage of root colonization is the movement of microbes to the plant root surface. Bacterial movement can be passive, via soil water fluxes, or active, via specific induction of flagellar activity by plant-released compounds (chemotaxis). The second step in colonization is 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 expression (Brimecombe *et al.*, 2001). PGPR could colonize the roots externally and to a lesser extent internally (Del Gallo & Fendrik, 1994; Mia *et al.*, 1999). Bacterial motility could contribute to survival in soil and the initial phase of colonization, where attachment and movement to the root surface are important (Turnbull *et al.*, 2001).

The secure attachment of PGPR is essential for a long-term association with the host plant roots for three reasons: (i) if the bacteria are not attached to root epidermal cells, substances excreted by the bacteria will diffuse into the rhizosphere and are consumed by nutritionally versatile microorganisms before reaching the plant, (ii) without a secure attachment, water may wash the bacteria away from the rhizosphere to perish in the surrounding, nutrient-deficient soil, (iii) association sites on roots with no attached PGPR are vulnerable to other aggressive, and possibly non-beneficial, colonizers (Bashan & Holguin, 1997).

PGPR strains showed variable patterns of root colonizations namely specific and non-specific the later can colonize a wide range of plant species (Bashan & Holguin, 1997). *Azospirillum* are known as good colonizers of cereals (Baldani *et al.*, 1986; Gafni *et al.*, 1986; Bashan *et al.*, 1986 & 1987; Murty & Ladha, 1987), vegetables (Hadas & Okon, 1987; Bashan *et al.*, 1989), oil palm (Amir, 2001) and mangrove plants (Puente *et al.*, 1999). However, root colonization pattern vary greatly among *Azospirillum* strains (Bashan *et al.*, 1990). A high level of bacterial colonization is found in the middle lamellae, an area of longitudinal contact between epidermal cells of the roots (Bowen & Rovira, 1976). In general, root caps of many plants are not colonized by bacteria (Foster & Bowen, 1982).

Bashan and Levanony (1990) reported that the preferred sites for colonization in most plant species studied are the elongation and root hair zones, where the bacteria form an aggregate type of colonization supported by massive fibrillar material. The colonization sites in some grasses correspond to the areas, where root mucigel is present. The area around the point of emergence of lateral roots usually shows high colonization (Bilal *et al.*, 1993). Different strains of PGPR show different patterns of colonization. Assmus *et al.* (1995) suggested that *A. brasilense* Sp7 was restricted to the root hair zone, while *A. brasilense* Sp-245 was found repeatedly at high density in the interior of root hair cells. *A. halopraeferens* was a better root surface colonizer than *A. brasilense*, which colonized

the entire root of black mangrove seedlings (Puente *et al.*, 1999). Bashan *et al.* (1993) reported that there are at least two different types of anchoring in *Azospirilla*: a weak attachment to a non-biological surface and a stronger attachment to roots. The polar flagellum of *A. brasilense*, which is primarily used for swimming, is involved in the attachment process of the bacteria to roots. Inoculation of *Azospirillum* sp. strain Sp7 and *Bacillus* sp. Electron micrographs from the root colonization study in banana tissue cultured plantlets, conducted by Mia (2002), clearly demonstrated that PGPR stains *A. brasilense* (Sp7) and *Bacillus sphaericus* (UPMB10) strains could successfully form colonies on the banana roots (Plate 2). Bacterial colonization by Sp7 and UPMB10 occurred mainly on the root surface and more cells were found in the root hair proliferation zone, while the root hair *per se* is devoid of bacteria. Strain Sp7 preferentially colonized the root hair proliferation zone with a few cells found on the root elongation and root cap zone. Occurrence of more cells of Sp7 in the root hair zone was due to the presence of fibrillar matrix, which aided in the attachment of bacterial cells on the root surface. But strain UPMB 10 was capable of efficiently colonizing all the three root sections (cap, elongation & hair proliferation zone). The presence of more UPMB10 cells throughout the whole roots indicated that this strain was able to utilize exudates produced by the three zones. Similar findings of root colonization pattern have been shown by several investigators (Assmus *et al.*, 1995; Bashan & Holguin, 1997), who concluded that colonization of PGPR corresponded to the areas, where root mucigel was present. *A. brasilense* strain Sp7 preferred to colonize the root hair zone of wheat seedlings (Levanony *et al.*, 1989). Saubidet and Barneix (1998) have shown that inoculation with different strains of *A. brasilense* to young wheat plants produced successful colonization of both the rhizoplane and endorhizosphere and stimulated the root and shoot growth.

Recently, endophytic N₂-fixing bacteria have been introduced. These diazotrophs colonize roots, stems and leaves of cereals endophytically, where they probably suffer less competition from other microorganisms for carbon substrates than rhizosphere bacteria and possibly excrete part of their fixed N₂ directly into the plants (Baldani *et al.*, 2000). Lower and higher plants have endophytes that can be found intercellularly, *Cyanobacterium flavescens* and *Bacillus pumilus* could colonize intercellularly in rice seedlings, where they influence the growth and development of host plants (Bacilico-Jimenez, 2001). PGPR colonizes plant roots externally and internally (Patriquin *et al.*, 1983; Okon, 1985).

Plant growth and development: Bacteria that can increase plant growth and productivity have been known for over a century (Brown, 1974). Host plants are benefited by improved root development and sub-sequent increase in rates of water and mineral nutrient uptake (Okon & Kapulnik, 1986). *Azospirillum* inoculation in wheat has been shown to result in enhanced cell division in the root

tips (Levanony & Bashan, 1989), while in maize it increased diameter and length of lateral roots (Hartmann *et al.*, 1983) and promoted root hair development and branching, which caused alteration in arrangement of root cortex cells (Kapulnik & Okon, 1983). The PGPR inoculation also increased the root hair number, length and density. Similar inoculation on wheat caused fork-like deformation of root hairs and stimulated the formation of adventitious roots (Fallik *et al.*, 1994).

PGPR exhibits a strain specific effect on root hair deformation in wheat similar to the strain-specific effect of *Rhizobium* on root hair deformation for nodule formation in legumes (Patriquin *et al.*, 1983). However, inoculation of PGPR enhanced the appearance of root hairs and increased the root length in tomato seedlings (Hadas & Okon, 1987). Lin *et al.* (1983) and Kapulnik *et al.* (1985b) found changes in the external layers of root cortex in maize and wheat seedlings. They also caused asymmetric growth of the root tip and stimulated root hair formation (Morgenstern & Okon, 1987). Moreover, it has been proposed that PGPR affects root branching and development in inoculated pearl millet and increases the number of lateral roots and root hairs without changing the root dry weight, probably by producing phytohormones such as cytokinins, gibberellins and auxin (Tien *et al.*, 1979).

Growth-promoting effects of PGPR inoculation are mainly derived from morphological and physiological changes and enhancement of water and mineral nutrient uptake as observed in inoculated sorghum plant roots (Sarig *et al.*, 1988). Murty and Ladha (1988) found that inoculation of *A. lipoferum* to rice roots reduced root length significantly and caused significant increases in shoot fresh and dry weights, but had no effect on root surface area. *Azospirillum* inoculation increased dry matter by 40% in *Zea mays* and in *Triticum aestivum* (Bashan, 1998). Similarly, nitrite, added directly or excreted by *Azospirillum* in nitrate respiration, causes a sharp increase in the lateral root formation in wheat (Bothe *et al.*, 1992). Beneficial effects of host plants by PGPR inoculation may be due to increased uptake of combined N from the soil (Kapulnik *et al.*, 1985b). Such an effect has been observed for NO₃⁻ in *Azospirillum*-inoculated maize root segments (Lin *et al.*, 1983) in wheat grown under hydroponics system (Kapulnik *et al.*, 1985a) and in sorghum grown under field condition (Sarig *et al.*, 1984). These results showed that graminaceous plants are potentially capable of establishing an association with diazotrophic bacteria in which the ammonium-excreting bacteria provide the host plants with a source of nitrogen.

Inoculation of oilseed (*Salicornia bigelovii* Torr.) with PGPR has been reported to increase plant biomass, palmitic acid, total N and protein content. The PGPR inoculation also increased P content through P solubilization (Bashan *et al.*, 2000).

Inoculation of rhizobacteria *viz.* *Pseudomonas fluorescens*, *Azotobacter cryococcum* and *A. brasilense* alone and in combination with root symbionts, *Rhizobium*

sp. and *Glomus mossae* improved plant growth and reduced gall formation on chick pea (*Cicer arietinum*) (Siddique & Mahmood, 2001). The effect of the combined inoculation of the arbuscular mycorrhizal fungus *Glomus manihotis* and a rhizobacteria consortium of *Bacillus* spp. on micro-propagated banana plantlets, during the acclimatization phase under routine nursery conditions was investigated by Rodríguez-Romero (2005). Micropropagated banana plantlets from the 'Grande Naine' cultivar were inoculated with mycorrhiza and rhizobacteria either alone or combined and the results showed that combined inoculated plants showed growth parameters i.e., total fresh weight, aerial dry weight, shoot length and leaf area, significantly higher than non-treated control bananas. Leaf mineral content i.e., N, P and K was also significantly increased following combined application of both microorganisms. No adverse effect on mycorrhizal colonization due to *Bacillus* spp. inoculation could be detected. The plant growth promoting effects of PGPR are mainly derived from morphological and physiological changes in roots of inoculated plant (Okon *et al.*, 1988; Sarig *et al.*, 1988). The inoculation process could stimulate the root growth and development in bananas as reported by Mia (2002). The results showed that increased root growth in bananas occurred almost in all dimensions namely production of primary (1^0) and secondary (2^0) roots, longer roots (42%) and greater volume (48%) and mass (43%). Inoculation stimulated the reproductive growth as shown by early flowering (3 weeks) and increased bunch yield (51%) (Fig. 1, Plate 3). The earliness of flowering is attributed to early development of plants with efficient nutrient and water uptake. The study reflected an additional 3 weeks saving in the maturation period of bananas. This indicated that PGPR stimulated plants for early reproductive development through enhanced nutrient uptake. Similar results have been also demonstrated by Tiwary *et al.* (1998), who found that inoculation of banana sucker with *Azospirillum* twice (sucker + soil inoculation) resulted in maximum plant height and leaf size in plants receiving 50% of the recommended dose of nitrogen. However, inoculation of *Azospirillum* had no significant response in reducing the time required for shooting from planting. *Azospirillum* inoculated plants produced higher number of hands/bunch, compensated 50% of the recommended dose of nitrogen and the number of hands/bunch obtained was at par with double inoculation. Inoculation with *Azospirillum* produced maximum yield of banana (69.15 t ha^{-1}). A fairly high T.S.S. and reduced sugar content were recorded with *Azotobacter* inoculated plants. However, they did not find any consistent results on total sugar and acidity content of fruit.

Nitrogen fixation and utilization: Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with PGPR. This was mainly because of an increase in number of nitrogenous compound and nitrogenase activity in inoculated plants (Bashan & Holguin, 1997). Furthermore, research using N-balance, ^{15}N isotope dilution and ^{15}N natural abundance studies have

provided strong evidence that some tropical grasses, especially sugar cane (*Saccharum* spp.), wetland rice (*Oryza sativa*) and kallar grass (*Leptochloa fusca*) can obtain at least part of their N-needs from biological nitrogen (N_2) fixation (BNF) (James, 2000). PGPR inoculation in bananas showed a significant amount of nitrogen fixation (Mia *et al.*, 2007) and N_2 -fixing bacteria were isolated from banana (*Musa* spp.) and pineapple [*Ananas comosus* (L.) Merrill]. They were characterized by amplified 16S ribosomal DNA restriction analysis and 16S rRNA sequence analysis. *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Burkholderia brasiliensis* and *B. tropicalis* were identified. Eight other types were placed in close proximity to these genera and other alpha and beta *Proteobacteria* (Cruz, 2001) and constituted a minor portion of banana endophytic bacteria (Martinez *et al.*, 2003). Nitrogen fixation associated with roots of grasses has been recognized as an important component of the nitrogen cycle in a range of ecosystems (Chalk, 1991). 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. PGPR can contribute about 31% and 86% of the total N requirement through BNF in maize and oil palm seedlings, respectively, under *in vitro* condition (Shamsuddin, 1994 & El Kommy *et al.*, 1998).

The effect of some PGPR strains on plants is not solely via N_2 fixation rather due to increase in nitrate assimilation. *Azospirillum* stimulate nitrate reduction in roots and thus decrease nitrate translocation to the leaves, while inoculation with NR mutant cause direct translocation and reduction of nitrate in the plant foliage. It is impossible to say with certainty that the observed effects (if any) of such inoculation are due to plant-associated N_2 fixation and not to other factors, such as hormonal effects, enhanced scavenging of N from the soil due to bacterial-induced increase in root growth and/or bacterial nitrate reductase (Michiels *et al.*, 1989; Bashan & Levanony, 1990; Sumner, 1990). However, by evaluating world-wide data accumulated over the past 40 years on greenhouse and field inoculation experiments with PGPR it can be concluded that these are capable of promoting the yield of agriculturally important crops in different soils and climatic regions. The data from field experiments indicate 60 to 70% occurrence of success with statistically significant yield increase in the order of 5 to 30% (Okon & Labandera-Gonzales, 1994). However, a study on N_2 fixation by ^{15}N isotopic dilution technique with 45 days old banana seedlings conclusively indicated that rhizobacterial inoculation with 33% fertilizer-N (50 ppm) could fix 8.85 to 9.69 mg N plant $^{-1}$ (5.0-5.3% Ndfa) (Fig.2). 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 (Mia *et al.*, 2007). **Nutrient uptake:** Enhanced mineral uptake in inoculated cereal plant was proposed as a possible mechanism of plant growth enhancement by PGPR. The major element involved

Fig. 1. Effect of PGPR strains Sp7 and UPMB10 inoculation on bunch yield and days to flowering of bananas

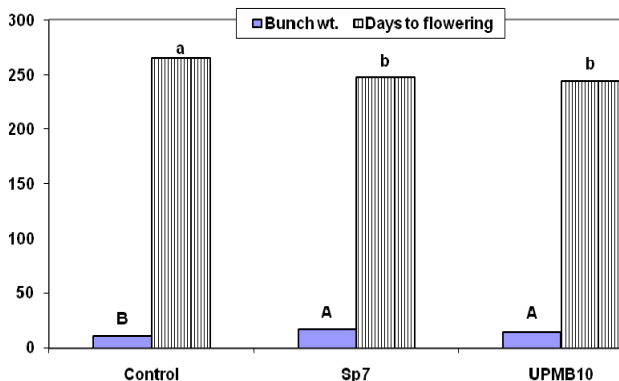
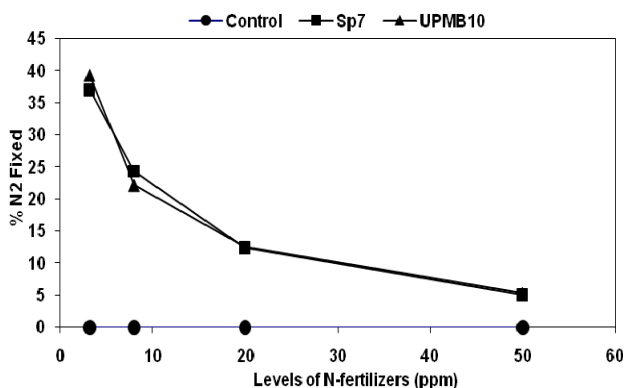


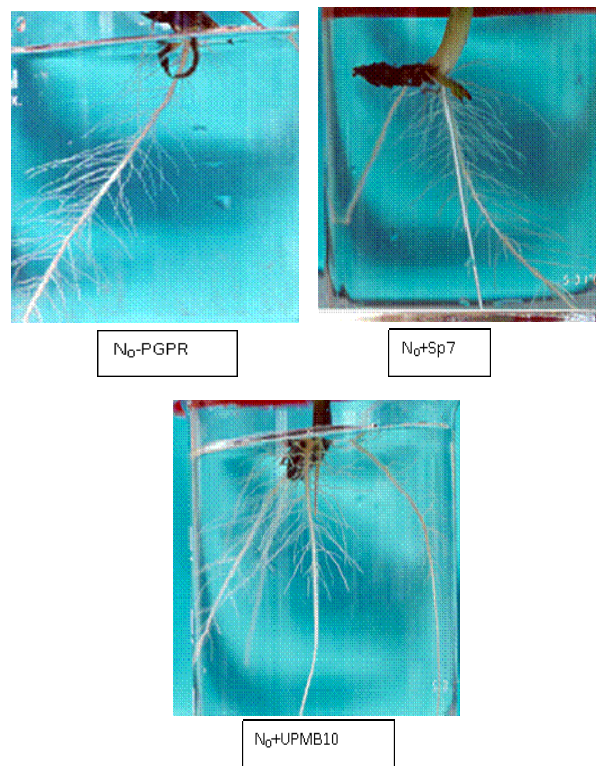
Fig. 2. Percentage of N₂ fixed by PGPR strains Sp7 and UPMB10 in association with banana roots



was suggested to be N in the form of nitrate in wheat, sorghum and corn plants (Lin *et al.*, 1983; Kapulnik *et al.*, 1985 a; Ferreira *et al.*, 1987; Boddey & Dobereiner, 1988; Kucey, 1988) or ammonium in rice plants (Murty & Ladha, 1988). Other elements such as P and K were also suggested to play a key role in this plant-bacterium interaction. Shamsuddin *et al.* (2000) found increased amounts of P and K uptake in banana plants inoculated with PGPR. Combined inoculation of *A. brasilense* and the phosphate-solubilizing bacteria *Pseudomonas strica* or *Bacillus polymyxa* on field-grown sorghum significantly increased grain and dry matter yields and N and P uptake as compared with single inoculation of individual organisms (Alagawadi & Gaur, 1992). Combined application of *Azospirillum*, *Azotobacter* and inorganic nitrogen fertilizer resulted in taller plants, number of leaves and girth, but could not show yield improvement and root development in bananas compared to un-inoculated control (Wange & Patil, 1994). Inoculation of *A. brasilense* increased the dry weight, plant height, P absorption and lipid content in oil seed (Bashan *et al.*, 2000).

Improved root growth and function were proposed in the late 1970s as a possible mechanism by which PGPR affects plant growth (Fallik *et al.*, 1994). The presence of root hairs has been shown to increase the surface area of a

Plate 1. Roots of banana plantlets 7 days after inoculation with PGPR strains Sp7 and UPMB10 grown under hydroponics condition



root system 5-18 times (Dittmer, 1937); consequently, plant scientists have assumed that root hairs aid in ion uptake by roots. Bray (1954) has indicated that although the entire soil volume of the rooting zone could be involved in supplying mobile nitrates, only a limited soil volume at the soil-root interface will be involved in supplying relatively immobile and highly adsorbed ions such as P. Inoculation of *A. brasilense* on sorghum (*Sorghum bicolor*), wheat and corn has resulted in a significant enhancement of NO₃⁻, K⁺, P uptake by the host plant. Rai and Hunt (1993) observed the occurrence of associative N₂-fixation and higher mineral nutrient uptake (P, K, Ca, Mg) and growth in maize, when inoculated with *Azospirillum* spp. in both sterilized and non-sterilized saline calcareous soils. Mixed inoculation of *Vicia faba* L. with four different PGPR strains changed the total accumulation, concentration and/or distribution of the macro-and micronutrients (Rodelas *et al.*, 1999).

The increase of nutrients observed in this system seemed to be due to a general increase in root area and not to a specific uptake rate. *A. brasilense* may improve the efficiency of applied mineral nutrients by helping the plant scavenge limiting nutrients, a role similar to that of mycorrhiza in the phosphate recovery. The PGPR inoculation changes many root and shoot parameters. These changes are directly attributed to positive bacterial effects on mineral uptake by the plant. Enhancement in uptake of NO₃⁻, NH₄⁺, HPO₄²⁻, K⁺, Rb⁺ and Fe²⁺ by *Azospirillum* (Lin

Plate 2. Banana root surface colonization of PGPR strain *Bacillus sphaericus* (strain UPMB10) isolated from oil palm roots

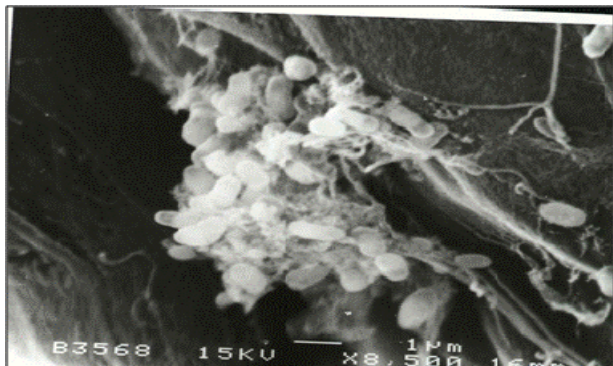
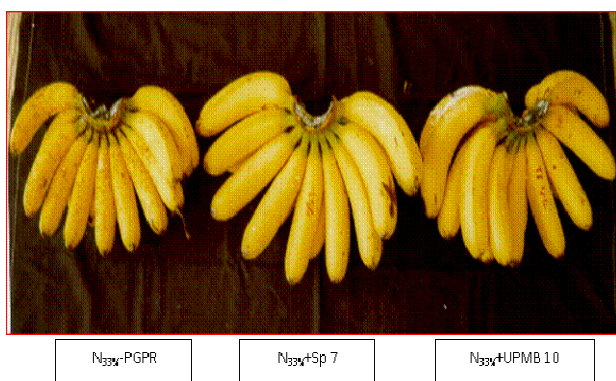


Plate 3. Hands and fingers of banana plants inoculated with PGPR strains Sp7 and UPMB10 and 33% fertilizer-N compared to uninoculated control



et al., 1983; Jain & Patriquin, 1984; Kapulnik *et al.*, 1985a; Barton *et al.*, 1986; Morgenstern & Okon, 1987; Murty & Ladha, 1988; Sarig *et al.*, 1988) was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves. Soil and rhizosphere bacteria can affect the mineral nutrition of plants by changing root-uptake characteristics of different crop plants. However, other researchers have shown that enhanced growth of wheat and soybean was not necessarily because of a general enhancement in mineral uptake (Bashan *et al.*, 1990).

Many workers suggested that root hairs improve the P uptake capacity of a plant (Lauchil, 1967; Drew & Nye, 1969), some doubt this. Bole (1973) found that P uptake was enhanced not as a consequence of root hair proliferation, but through increasing the ion selectivity. However, PGPR inoculation has been shown to successfully enhance the P uptake in oilseed and banana (Bashan *et al.*, 2000; Shamsuddin *et al.*, 2000).

PGPR inoculation has been shown to improve mineral uptake in several cereal species (Lin *et al.*, 1983; Kapulnik *et al.*, 1985a; Morgenstern & Okon, 1987; Murty & Ladha, 1988; Sarig *et al.*, 1988; Bashan *et al.*, 1990) and to enhance proton efflux from roots of wheat seedlings. Proton efflux, the excretion of H^+ from the roots is one of the important

mechanisms of ion uptake. This enhancement lasted for several days and in-oculation partially reverses proton efflux inhibited by ATP-ase inhibitor (Bashan *et al.*, 1989). It is not clear how bacterial colonization of roots affects cell membranes. This is of importance since mineral uptake is usually closely related to membrane activity. The capacity of an intact plant to lower the pH of its rhizosphere is exhibited by several species. PGPR strain *A. brasilense* Cd inoculation can affect membrane activity and proton efflux of wheat root, which requires high metabolic activity of both participants in the plant-bacteria association and may be involved in increasing mineral uptake of *Azospirillum*-inoculated plants (Bashan, 1990). Inoculation of soybean seedlings with *A. brasilense* Cd has been shown to significantly reduce the membrane potential in every root part and this reduction was greatest in the root elongation zone (Bashan, 1991b; Bashan & Levanony, 1991). Inoculation of soybean and cowpea with *A. brasilense* Cd increased proton efflux from their roots and changed the phospholipid content in cowpea plant membranes (Bashan *et al.*, 1992).

Enhancement of physiological properties: PGPR inoculation increased the physiological properties of the host plants namely, photosynthetic (Mia *et al.*, 2000) rate and stomatal conductance, but reduces proline accumulation. *A. brasilense* inoculated sorghum plants has been shown to improve leaf water potential under field condition (Fallik *et al.*, 1994). The water regime of sorghum plants was improved by inoculation as observed by their higher leaf water potential, lower canopy temperature and greater stomatal conductance and transpiration (Sarig *et al.*, 1988).

PGPR inoculation increased the stomatal conductance and lowered the proline concentration in leaves of banana seedling grown under submerged condition. It also alleviated plant stress due to submergence, while leaf growth and chlorophyll content and new root formation were lowered under submerged condition (Shamsuddin *et al.*, 1999).

Photosynthetic capacity of N_2 -fixing bacteria was higher compared to N user, since the former needed more photosynthate to meet the higher demand by diazotrophs during the N_2 -fixing process (Quilici & Medina, 1998). Strong sink strength of inoculated roots has been shown to induce an increase in source leaf photosynthesis of soybean plants (De Veau *et al.*, 1990). *Azospirillum* and rhizobacterial inoculation increased the photosynthetic rate of oil palm seedlings (Amir *et al.*, 2001).

Biocontrol: Plant-growth-promoting rhizobacteria have recently been shown to induce systemic resistance against fungi, bacteria and viruses, as well as to enhance plant growth. Combined application of arbuscular mycorrhizal fungi and PGPR highly benefits banana plants and therefore, could be considered during the acclimatization stage of micropropagated banana (Rodríguez-Romero, 2005). The inoculation in banana nursery showed better growth and seedling health and consequently increased the seedling survival rates (Jaizme-Vega *et al.*, 2005). Plant

tissues produce ethylene under the environmental stress, including stress caused by drought and high salt. An increased concentration of ethylene in plants can cause inhibition of plant growth. The ACC (1-aminocyclopropane-1-carboxylic acid) deaminase produced by PGPR can reduce the plant's ethylene concentration by cleaving the ethylene precursor ACC, resulting stimulated plant growth by reducing environmental salt and drought stress (Lim & Kim, 2009).

CONCLUSION

This review has been summarized the current information on PGPR, its application on bananas, as well as different crop plants and indicates the potential of PGPR as a bioenhancer and biofertilizer for banana cultivation. Nitrogen fixation, plant growth promotion and improved nutrient absorption are important criteria for achieving a sustainable banana production system. During the last four decades, PGPR research has brought together scientists from multiple disciplines endeavoring a wide range of topics including: discovery of novel PGPR strains and traits; performance in greenhouse and field trials; production, formulation and delivery of inoculum; mechanisms of growth promotion and biocontrol and their molecular and biochemical basis; root colonization and rhizosphere competence traits; role of PGPR in suppressive soils; plant, pathogen and rhizosphere community responses to PGPR and recombinant PGPR and risk assessment. Now a new chapter in PGPR research has began with the genome sequencing of several well-studied strains (Weller *et al.*, 2009).

However, there are some limitations of using PGPR in banana production system of inconsistent results in the field. Despite the recent advances, commercialization of this technology demands extensive optimization and comprehensive study of the aftereffects of the application.

REFERENCES

- Abdullah, M.Y., N.M. Hassan, Z. Mahmood and Z. Talib, 1999. Trend in foliar nutrient concentrations and contents and its implication on leaf area index development and yield in banana cultivar 'berangan'. In: Zakaria, W., T.M.M. Mahmud, D. Siti Khalijah, M.F. Nor Aini and M. Marziah (eds.), *Proc. First National Banana Seminar*, pp: 95–105. Awana, Genting, Pahang, Malaysia
- Alagawadi, A.R. and A.C. Gaur, 1992. Inoculation of *A. brasilense* and phosphate-solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land. *Tropical Agric.*, 69: 347–350
- Amir, H.G., Z.H. Shamsuddin, M.S. Halimi, M.F. Ramlan and M. Marziah, 2001. Effects of *Azospirillum* inoculation on N₂ fixation and growth of oil palm plantlets at nursery stage. *J. Oil Palm Res.*, 13: 42–49
- Assmus, B., P. Hutzler, G. Kirchhof, R. Amann, J.R. Lawrence and A. Hartmann, 1995. *In situ* localization of *Azospirillum brasilense* in the rhizosphere of wheat with fluorescently labelled, rRNA-targeted oligonucleotide probes and scanning confocal laser microscopy. *Appl. Environ. Microbiol.*, 61: 1013–1019
- Bacilico-Jimenez, 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, J.I., L. Caruso, V.L.D. Baldani, S.R. Goi and J. Dobereiner, 1997. Recent advances in BNF with non-legume plants. *Soil Biol. Biochem.*, 29: 911–922
- Barton, L.L., G.V. Johnson and S. Orbockmiller, 1986. The effect of *A. brasilense* on iron absorption and translocation by sorghum. *J. Plant Nutr.*, 9: 557–565
- Bashan, Y., 1990. Short exposer to *A. brasilense* Cd inoculation enhanced proton efflux of intact wheat root. *Canadian J. Microbiol.*, 36: 419–425
- Bashan, Y., 1998. Inoculants of plant growth promoting rhizobacteria for use in agriculture. *Biotechnol. Adv.*, 16: 729–770
- Bashan, Y., L. Aclarz-Melendez and G. Toledo, 1992. Response of soybean and cowpea root membranes to inoculation with *A. brasilense*. *Symbiosis*, 13: 217–228
- Bashan, Y., G. Holguin and R. Lifshitz, 1993. Isolation and characterization of plant-growth promoting rhizobacteria. In: Glick, B.R. and J.E. Thompson (eds.), *Methods in Plant Molecular Biology and Biotechnology*, pp: 313–345. CRC Press, Boca Raton, Florida
- Bashan, Y., H. Levanony and E. Klein, 1986. Evidences for a weak active external adsorption of *A. brasilense* Cd to wheat roots. *J. Gen. Appl. Microbiol.*, 132: 3069–3073
- Bashan, Y. and H. Levanony, 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Canadian J. Microbiol.*, 36: 591–608
- Bashan, Y., H. Levanony and O. Ziv-Vecht, 1987. The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season. *Canadian J. Microbiol.*, 33: 1074–1079
- Bashan, Y., M. Moreno and E. Troyo, 2000. Growth promotion of seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. *Biol. Fertil. Soils*, 32: 265–272
- Bashan, Y., H. Levanony and E. Klein, 1986. Evidences for a weak active external adsorption of *A. brasilense* Cd to wheat roots. *J. Gen. Appl. Microbiol.*, 132: 3069–3073
- Bashan, Y., H. Levanony and O. Ziv-Vecht, 1987. The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season. *Canadian J. Microbiol.*, 33: 1074–1079
- Bashan, Y., H. Levanony and G. Mitiku, 1989. Changes in proton efflux in intact wheat roots inoculated by *A. brasilense* Cd. *Canadian J. Microbiol.*, 35: 691–697
- Bashan, Y., H. Levanony and E. Klein, 1986. Evidences for a weak active external adsorption of *A. brasilense* Cd to wheat roots. *J. Gen. Appl. Microbiol.*, 132: 3069–3073
- Bashan, Y., 1998. Inoculants of plant growth promoting rhizobacteria for use in agriculture. *Biotechnol. Adv.*, 16: 729–770
- Bashan, Y., S.K. Harrison and R.E. Whit Moyer, 1990. Enhanced growth of wheat and soybean plants inoculated with *A. brasilense* is not necessarily due to general enhancement of mineral uptake. *Appl. Environ. Microbiol.*, 56: 769–775
- Bashan, Y. and G. Holguin, 1997. *Azospirillum*/plant relationship: environmental and physiological advances, 1990–1996. *Canadian 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
- Bray, R.H., 1954. A nutrient mobility concept of soil-plant relationships. *Soil Sci.*, 78: 9–22
- Brimecombe, J.M., F.A. De Leij and J.M. Lynch, 2001. The effect of root exudates on rhizosphere microbial population. In: Pinton, R., Z. Varanini and Nannipieri (eds.), *The Rhizosphere*, pp: 95–140. Marcel Dekker Inc., New York, USA
- Bilal, R., G. Rasul, M. Arshad and K.A. Malik, 1993. Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter* spp. on root surface of grasses. *World J. Microbiol. Technol.*, 9: 63–69
- Boddey, R.M., O.C. De Oliveira, S. Urquiaga, V.M. Reis, F.L. Olovaes, 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. and J. Dobreiner, 1988. Nitrogen fixation associated with grasses and cereals: recent results and perspective for future research. *Plant Soil*, 108: 53–65
- Bole, J.B., 1973. Influence of root hairs in supplying soil phosphorus to wheat. *Canadian J. Soil Sci.*, 53: 169–175
- Bowen, D.G. and A.D. Rovira, 1976. Microbial colonization of plant roots. *Annual Rev. Phytopathol.*, 14: 121–144
- Chalk, P.M., 1991. The contribution of associative and symbiotic nitrogen fixation to the nitrogen nutrition of non-legumes. *Plant Soil*, 132: 29–39
- Cocking, E.C., 2000. Helping plants get more nitrogen from air. *European Rev.*, 8: 193–200
- Cruz, L.M., E.M. De Souza, O.B. Weber, J.I. Baldani, J. Dobreiner and F.O. Pedrosa, 2001. 16S Ribosomal DNA Characterization of Nitrogen-Fixing Bacteria Isolated from Banana (*Musa* spp.) and Pineapple [*Ananas comosus* (L.) Merrill]. *Appl. Environ. Microbiol.*, 67: 2375–2379
- De Langhe, E.R., R. Swennen and G.F. Wilson, 1983. Aspects hormoaux rejectionage des bananiers plantains. *Fruits*, 38: 318–325
- De Veau, E.J., J.M. Robinson, R.D. Warmbrodt and P. Van Berkum, 1990. Photosynthesis and photosynthate partitioning in N₂-fixing soybeans. *Plant Physiol.*, 94: 259–267
- Del Gallo, M. and I. Fendrik, 1994. The Rhizosphere and *Azospirillum*. In: Okon, Y. (ed.), *Azospirillum/Plant Associations*, pp: 57–85. CRC Press, London
- Dittmer, H.J., 1937. A quantitative study of the root hairs of a winter rye plant (*Secle cereale*). *American J. Bot.*, 24: 417–420
- Dobreiner, J., 1995. Isolation and identification of aerobic nitrogen fixing bacteria from soil and plants. In: Alef, K. and P. Nanniperi (eds.), *Methods in Applied Soil Microbiology and Biochemistry*, pp: 134–141. Academic press, London
- Dobreiner, J., 1997. Biological nitrogen fixation in the tropics: social and economic contributions. *Soil Biol. Biochem.*, 29: 771–774
- Drew, M.G. and P.H. Nye, 1969. The supply of nutrient ions by diffusion to plant roots in soil II. The effect of root hairs on the uptake of K by roots of ryegrass. *Plant Soil*, 31: 407–424
- El-Komy, H.M.A., T.M.M. Moharram and M.S.A. Safwat, 1998. Effects of *Azospirillum* inoculation on growth and N₂ fixation of maize subjected to different levels of FYM using ¹⁵N-dilution method. In: Malik, K.A. (ed.), *Nitrogen Fixation with Non-legumes*, pp: 49–59. Kluwer Academic Publishers, London
- Fallik, E., S. Sarig and Y. Okon, 1994. Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon, Y. (ed.), *Azospirillum/Plant Associations*, pp: 77–85. CRC Press, Florida
- Ferreira, C.B., M.S. Fernandes and J. Dobreiner, 1987. Role of *A. brasilense* nitrate reductase in nitrate assimilation by wheat plants. *Biol. Fertil. Soil*, 447–453
- Foster, R.C. and G.D. Bowen, 1982. In: Lacy, G.H. (ed.), *Plant Surfaces and Bacterial Growth: The Rhizosphere, Phytopathogenic Prokaryotes*, pp: 159–185. Academic Press, New York
- Gafni, R., Y. Okon, Y. Kapulnik and M. Fischer, 1986. Adsorption of *A. brasilense* to corn roots. *Soil Biol. Biochem.*, 18: 69–76
- Guerra, M.S.P. and C. Jaizme-Vega, 2005. Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agron. Sustain. Develop.*, 25: 395–399
- Jaizme-Vega, M.C., A.S. Rodriguez-Romero and M.S.P. Guerra, 2004. Potential use of rhizobacteria from the *Bacillus* genus to stimulate the plant growth of micropropagated bananas. *Fruits*, 59: 83–90
- James, E.K., 2000. Nitrogen fixation in endophytic and associative symbiosis. *Field Crop Res.*, 65: 197–209
- Hadas, R. and Y. Okon, 1987. Effect of *A. brasilense* on root morphology and respiration in tomato seedlings. *Biol. Fertil. Soils*, 5: 241–247
- Haque, S.E. and A. Ghaffar, 1993. Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. *J. Phytopathol.*, 138: 157–163
- Hartmann, A., M. Singh and W. Klingmuller, 1983. Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. *Canadian J. Microbiol.*, 29: 916–923
- Kapulnik, Y., 1991. Non-symbiotic nitrogen fixing microorganisms. In: Waisel, Y., A. Eshel and U. Kafkafi (eds.), *Plant Roots: The Hidden Half*, pp: 703–716. Marcel Dekker Inc., New York
- Kapulnik, Y. and Y. Okon, 1983. Benefits of *Azospirillum* inoculation on wheat: effects on root development, mineral uptake, nitrogen fixation and crop yield. In: Klingmuller, W., (ed.), *Azospirillum* II., pp: 163–170. Birkhauser Verlag, Stuttgart, Germany
- Kapulnik, Y., Y. Okon and Y. Henis, 1985b. Changes in root morphology of wheat caused by *Azospirillum* inoculation. *Canadian J. Microbiol.*, 31: 881–887
- Kloepper, J.W., J. Leong, M. Teintze and M.N. Schorth, 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 system and nitrogen fixation by associative nitrogen fixing bacteria measured under field conditions. *Canadian J. Microbiol.*, 34: 735–739
- Ladha, J.K., F.J. De Bruijn and K.A. Malik, 1997. Assessing opportunities for nitrogen fixation in rice: a frontier project. *Plant Soil*, 194: 1–10
- Ladha, J.K. and P.M. Reddy, 1995. Extension of nitrogen fixation to rice: necessity and possibilities. *Geo. J.*, 35: 363–372
- Lauchil, A., 1967. Investigation on the distribution and transport of ions in plant tissue with X-ray microanalyser I. Experiments on vegetative organs of *Zea mays*. *Planta*, 75: 185–206
- Lim, J.H. and S.D. Kim, 2009. *Plant Growth Promotion, Biological Control and Drought Stress Resistance Induction by Indigenous Multi-Functional PGPR*, p: 45. Abstract 8th International PGPR workshop, Oregon State University, Oregon
- Lin, W., Y. Okon and R.W. Hardy, 1983. Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brasilense*. *Appl. Environ. Microbiol.*, 45: 1775–1779
- 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
- Martinez, L., J. Caballero-Mellado, J. Orozco and E. Martinez-Romero, 2003. Diazotrophic bacteria associated with banana (*Musa* spp.). *Plant Soil*, 257: 35–47
- 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, 1998. Root stimulation and nutrient uptake of banana inoculated with *Azospirillum brasilense* and grown under hydroponic condition. In: Zakaria, W., T.M.M. Mahmud, S.D. Khalijah, M.F. Nor Aini and M. Marziah (eds.), *Proc. First National Banana Seminar*, pp: 122–133. Genting, Pahang, Malaysia
- 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. In: Vidyadaran, M.K., A.S.T.M. Tengku (eds.), *Proc. Eighth Scientific Conference of Electron Microscopy Society Malaysia*, pp: 173–174. Pahang, Malaysia
- Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2000. *Plant Growth Promoting Rhizobacteria for Sustainable Banana Production Under Hydroponics Condition*, pp: 78–80. International symposium on sustainable land management: paradigms for the new millennium, Kuala Lumpur, Malaysia
- Mia, M.A.B., 2002. Beneficial Effects of Rhizobacterial Inoculation on Nutrient Uptake, Growth and Yield of Banana (*Musa* spp.). *Ph. D. Thesis*, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
- Mia, M.A.B., Z.H. Shamsuddin, W. Zakaria and M. Marziah, 2002. Plant growth promoting rhizobacteria for banana production under hydroponics condition. In: Shamsuddin, J., J. Hamdan and A.W. Samsuri (eds.), *Sustainable Land Management*, pp: 185–190. Malaysian Society of Soil Science, Kuala Lumpur, Malaysia
- 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
- Michiels, K., J.D. Vanderleyden and A. Van Gool, 1989. *Azospirillum*-plant root association; a review. *Biol. Fertil. Soils*, 8: 356–368

- Morgenstern, E. and Y. Okon, 1987. Promotion of plant growth and NO_3^- and Rb^+ uptake in *Sorghum bicolor* x *Sorghum sudanense* inoculated with *A. brasilense*-Cd. *Arid Soil Res. Rehabilitation*, 1: 211–217
- Murty, M.G. and J.K. Ladha, 1987. Differential colonization of *Azospirillum lipoferum* on roots of two varieties of rice (*Oryza sativa* L.). *Biol. Fert. Soils*, 4: 3–7
- Murty, M.G. and J.K. Ladha, 1988. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponics conditions. *Plant Soils*, 108: 281–285
- Okon, Y., 1985. *Azospirillum* as a potential inoculant for agriculture. *Trends Biotechnol.*, 3: 223–228
- Okon, Y., Y. Kapulnik and S. Sarig, 1988. Field inoculation studies with *Azospirillum* in Israel. In: Suba Rao, N.S. (ed.), *Biological Nitrogen Fixation, Recent Developments*, p: 175. Oxford and IBH, New Delhi, India
- Okon, Y. and C.A. Labandera-Gonzales, 1994. Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biol. Biochem.*, 26: 1591–1601
- Phillips, D.A., 1980. Efficiency of symbiotic nitrogen fixation in legumes. *Annual Rev. Plant Physiol.*, 31: 29–49
- 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
- Patriquin, D.G., J. Dobreiner and D.K. Jain, 1983. Site and process of association between diazotrophs and grasses. *Canadian J. Microbiol.*, 29: 900–915
- Price, N.S., 1995. Banana morphology, Part 1: roots and rhizomes. In: Gowen, S. (ed.), *Banana and Plantains*, pp: 190–205. Chapman and Hall
- Quilici, A. and E. Medina, 1998. Photosynthesis-nitrogen relationships in pioneer plants of disturbed tropical montane forest sites. *Photosynthetica*, 35: 525–534
- Rai, R. and P.G. Hunt, 1993. Inoculation of maize varieties with salt tolerant mutants of *Azospirillum brasilense* and VAM fungi in saline calcareous soil. *Microbiol. Release*, 1: 243–251
- Reinhold, B. and T. Hurek, 1998. Interaction of graminaceous plants with *Azoarcus* spp. and their diazotrophic identification, localization and perspective to study their function. *Critical Rev. Plant Sci.*, 17: 29–54
- Robinson, J.C., 1996. *Bananas and Plantains*, pp: 172–174. CAB International, Wallingford, Oxon, UK
- Rodelas, B., J.G. Lopez, M.V. Toledo, C. Pozo and V. Salmeron, 1999. Influence of *Rhizobium/Azotobacter* and *Rhizobium/Azospirillum* combined inoculation on mineral composition of faba bean (*Vicia faba* L.). *Biol. Fertil. Soil.*, 29: 165–169
- Rodriguez-Romero, A.S., M.S. Guerra and M.D. Jaizme-Vega, 2005. Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agron. Sustainable Devel.*, 25: 395–399
- Saad, M.S., A.S.A. Shabuddin, A.G. Yunus and Z.H. Shamsuddin, 1999. Effects of *Azospirillum* inoculation on sweet potato grown on sandy tin-tailing soil. *Commun. Soil Sci. Plant Anal.*, 30: 1583–1592
- Sarig, S., A. Blum and Y. Okon, 1988. Improvement of water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. *J. Agric. Sci.*, 110: 271
- Saubidet, M.I. and A.J. Barneix, 1998. Growth stimulation and nitrogen supply to wheat plants inoculated with *Azospirillum brasilense*. *J. Plant Nutri.*, 21: 2565–2577
- Shamsuddin, Z.H., 1994. Application of microbial inoculant for crop production. In: *FFTC/RDA International Seminar on 'The Use of Microbial and Organic Fertilizers in Agricultural Production'*, pp: 1–13. Suweon, Korea
- Shamsuddin, Z.H., H.G. Amir, M.A.B. Mia, M.S. Halimi, W. Zakaria and M. Marziah, 1998. *Azospirillum* as a bioenhancer and biofertilizer for banana and oil palm seedlings. In: Yoshida, T., M. Pornchai, E.D. Cruz, E. Sukara and M.A.K.M. Ismail (eds.), *Biotechnology for Sustainable Utilization of Biological Resources in the Tropics*, Vol. 13, pp: 326–338. Osaka, Japan
- Shamsuddin, Z.H., H.G. Amir, M.A.B. Mia, M.S. Halimi, W. Zakaria and M. Marziah, 1999. Symbiotic and associative N_2 fixation with vegetable soybean, oil palm and bananas. In: Yoshida, T., T. Seki, M. Pornchai, R.V. Ebor, E. Sukara and M.A.K.M. Ismail (eds.), *Biotechnology for Sustainable Utilization of Biological Resources in the Tropics*, Vol. 14, pp: 102–118. Osaka, Japan
- Shamsuddin, Z.H., H.G. Amir, M.A.B. Mia, P. Premalatha, M.S. Halimi, S.K. Khor, M. Marziah, A.B. Arif and T.C. Ooi, 2001. Commercial production of biofertilizer and bioenhancer using *Azospirillum* and *Bacillus* spp. for improved growth of oil palm seedlings and bananas. *Proc. Bio Thailand*, p: 129. Bangkok, Thailand
- Siddiqui, Z.A. and I. Mahmood, 2001. Effect of rhizobacteria and root symbionts on the reproduction of *Meloidogyne javanica* and growth of chickpea. *Biores. Technol.*, 79: 41–45
- Stoltzfus, J.R., R. So, R.P. Malarvithi, J.K. Ladha and F.D. De Bruijn, 1997. Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant Soil*, 194: 25–36
- Stover, R.H. and N.W. Simmonds, 1987. *Banana*. Longman Scientific and Technical, Longman
- Suslow, T.V., 1982. Role of root-colonizing bacteria in plant growth. In: Mount, M.S. and G.H. Lacy (eds.), *Phytopathogenic Prokaryotes*, Vol. 1, pp: 186–223. Academic Press, New York, USA
- Sumner, M.E., 1990. Crop response to *Azospirillum* inoculation. *Adv. Soil Sci.*, 12: 53–123
- Tiwary, D.K., M.A. Hasan, P.K. Chattopadhyay, 1998. Studies on the effect of inoculation with *Azotobacter* and *Azospirillum* on growth, yield and quality of banana. *Indian Agric.*, 42: 235–240
- Tien, T.M., H.G. Diem, M.H. Gaskins and D.H. Hubbel, 1979. Plant growth substances produced by *A. brasilense* and their effects on the growth of pearl millet (*Pennisetum americanum* L.). *Appl. Environ. Microbiol.*, 37: 1016–1024
- Triplett, E.W., 1996. Diazotrophic endophyte: progress and prospects for nitrogen fixation in monocots. *Plant Soil*, 186: 29–38
- Tumbull, 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
- Wange, S.S. and P.I. Patil, 1994. Effect of combined inoculation of *Azotobacter*, *Azospirillum* with chemical nitrogen on Basarai banana. *Madras Agric. J.*, 81: 163–165
- Weber, O.B., K.R.S. Teixeira, G. Kirchhof, J.I. Baldani and J. Dobreiner, 1995. Occurrence of Endophytic Diazotrophic Bacteria in Pine Apple (*Ananas sp.*) and Banana (*Musa sp.*). International Symposium on Sustainable Agriculture for the Tropics-The Role of Biological Nitrogen Fixation, Rio-de-Jenerio
- Weller, D., 2009. 8th International PGPR Workshop. Oregon State University, Oregon
- Yanni, Y.G., R.Y. Rizk, V. Corich, A. Squatini, K. Ninke, S. Philip-Hollingsworth, G. Orgambide, F.D. De Bruijn, J. Stoltzfus, D. Buckley, T.M. Schmidt, P.F. Mateos, J.K. Ladha and F.B. Dazzo, 1997. Natural endophytic associations between *Rhizobium leguminosarum* bv. *trifoli* and rice roots and assessment of its potential to promote rice growth. *Plant Soil*, 194: 99–114
- Yanni, Y.G., R.Y. Rizk, F.K.A. El-Fattah, A. Squartini, V. Corich, A. Giacomini, F. De Bruijn, J. Rademaker, J. Maya-Fores, P. Ostrom, M. Vega-Hernandez, R.I. Hollingsworth, E. Martinez-Molina, P. Mateos, E. Velaquez, J. Woperis, E. Triplett, M. Umali-Garcia, J.A. Anama, B.G. Rolfé, J.K. Ladha, J. Hill, R. Mujoo, P.K. Ng and F.B. Dazzo, 2001. The beneficial plant-growth promoting association of *Rhizobium leguminosarum* bv. *Trifolii* with rice roots. *Australian J. Plant Physiol.*, 28: 845–870

(Received 17 August 2009; Accepted 08 October 2009)