MMSU Science and Technology Journal
Vol. 4 No. 1/January-June 2014

ISSN: 2012 0060

ISOLATION, CHARACTERIZATION, AND EVALUATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM RHIZOSPHERES OF SUGARCANE (*Sacharum officinarum*) AND WILD LILY SP.

Dionisio S. Bucao and Diana Rose M. de Leon*

Abstract

The role of plant-growth promoting rhizobacteria (PGPR) in sustaining organic crop production system is indispensable. As such, this study characterized and evaluated the rhizobacteria isolated from the rhizospheres of wild lily sp. and sugarcane in the City of Batac, Ilocos Norte, Philippines. Initially, 15 bacterial isolates were purified and characterized morphologically. However, isolates with similar characteristics were discarded and only six were evaluated to determine their effect on the early growth of glutinous corn (*Zea mays* L.) through a pot experiment. Promising isolates were biochemically characterized to determine their classification.

Corn plants inoculated with bacterial isolates were significantly taller than the control from 14 to 28 days after planting (DAE). At 28 DAE, the heights of the corn plants inoculated with LR10 (44.50cm), SC5 (43.00cm), SC4 (42.50cm), LR9 (42.33), and LB1 (40.67cm) were comparable to those inoculated with LR (39.33cm) but significantly taller than the control (35.37cm). The total biomass of corn plants inoculated with LB1 produced the heaviest (3.20g) followed by those inoculated with LR1 (2.57g) and SC4 (2.40g). Results of biochemical characterization indicate that isolate LB1 is classified as *Paenibacillus polymyxa*, LR1 as *Bacillus subtilis*, and SC4 as *Bacillus subtilis*.

Keywords: *Plant growth promoting rhizobacteria, bacterial isolates, rhizosphere, Paenibacilus polymyxa, Bacillus subtilis*

**Corresponding Author:*
Current Address: MMSU-Research Directorate, City of Batac, Ilocos Norte
e-mail: dsbucao@yahoo.com

Introduction

Synthetic fertilizer is indispensable in providing adequate nutritional needs of crops to attain higher yields. However, with the goal of providing the needs of the fast-growing population in Asia, particularly in the Philippines, synthetic fertilizer and pesticide application needed in producing crops had increased tremendously causing groundwater contamination (Gumtang et al, 1999) and natural ecosystems degradation (Tilman et al, 2002). Prolonged use and excessive application of synthetic fertilizer destroys soil crumbs-mixture of humus and clay that resulted to a highly compacted soil with reduced drainage and air circulation due to depletion of organic materials and trace nutrients. Furthermore, the soil becomes more acidic, which jeopardizes the microorganisms that are both beneficial to soil health and eventually the plants.

As such, development of alternative technologies that would revitalize or rejuvenate soil health and sustain production of safe and quality food like organically-grown crops is imperative. Growing organic products is a holistic management system that avoids the use of synthetic fertilizers, pesticides, and genetically-modified organisms. Likewise, it minimizes air, soil, and water pollution and optimizes soil health and productivity of interdependent communities of plants, animals, and people (FAO, 2007). In implementing Republic Act 10068 (popularly known as the Organic Agriculture Act of 2010), all sectors are obliged to improve soil fertility, in order to increase farm productivity, reduce pollution and environmental destruction. However, its promotion had faced many challenges like inadequate production support, policy gaps, as well as fragmented and inadequate research and development among others. Thus, the need to develop inquiry-based technologies that produce organic inputs is crucial for the successful program implementation.

The roles of plant growth-promoting rhizobacteria (PGPR) in crop production are essentials. PGPR are free-living soil bacteria that, when inoculated on seedlings before planting, would effectively colonize the root system and then enhance the growth and yield of the host plants (Kumar et al, 2014).

Moreover, PGPR are commonly isolated in the plant rhizosphere, a narrow zone closely surrounding the roots (Sivasakthi, 2013) that is characterized by intense microbial activities stimulated by the root exudates. Afshan et al (2015) described various ways that have been hypothesized to elucidate how PGPR benefit the host plant. These include the ability to: a) manufacture plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinins, and gibberellins (Glick, 1995; Haque and Dave, 2005; Marques et al, 2010); b) increase asymbiotic N_2 fixation (Sahin et al, 2004; Khan, 2005); c) solubilize inorganic phosphate and mineralize organic phosphate and/ or other nutrients (Glick, 1995; Lazarovits and Nowak, 1997; Jeon et al., 2003); and d) antagonize the effect against phytopathogenic microorganisms by producing siderophores, the synthesis of antibiotics, enzymes, and/or fungicidal compounds, and competing with detrimental microorganisms (Dey et al, 2004; Lucy et al, 2004).

Meanwhile, plants such as wild lily and sugarcane are known for their resilience to biotic and abiotic stresses that might be due to the synergistic effect of the microbial communities associated with them such as *Psuedomonas* (Botelho and Mendonça-Hagler, 2006) and *Bacillus species*. Both of which are known for their biocontrol and biofertilizer properties.

The information generated herein regarding the functional diversity of PGPR associated with the rhizospheres of lily and sugarcane is crucial for understanding their ecological role and potential utilization in sustainable crop production.

Generally, the study isolated, characterized, and evaluated the growth promoting activity of bacteria isolated from the rhizospheres of sugarcane and wild lily. Specifically, it isolated plant growth-promoting rhizobacteria from the rhizospheres of sugarcane and wild lily; morphologically characterized the selected bacterial isolates; evaluated the plant growth-promoting activity of bacterial isolates on the early growth stage of corn; and biochemically characterized isolates having plant growth-promoting activity.

Methodology

Sample Collection, Isolation and Purification of Bacteria

Roots and bulbs of wild lily and roots of sugarcane were carefully collected by uprooting the root system and then placed in a plastic bag and cooler ready for transporting to the laboratory. Samples weighing 20g of the lily roots (LR), peels of the lily bulbs (LB), and sugarcane roots (SR) were taken after gently washing with distilled water. They were separately placed in 250mL flasks containing 90mL sterile distilled water and shaken (120rpm) in an orbit motion for 30min. Ten-fold serial dilutions for each sample were made and 0.1mL aliquots from the 10^{-6} to 10^{-10} were spread onto plates containing nutrient agar. The plates were incubated for 36hr at 30°C and only plates with 25-250 colonies were counted and considered for characterization (Breed and Dotterrer, 1916; Tomasiewicz et al, 1980). Five bacterial isolates from LR, LB, and SR were selected. Different colonies appearing on the medium were isolated for characterization such as size, color-pigmentation, form, margin, elevation, surface, texture, and opacity as described by Somasegaran and Hoben (1994). The Gram reaction was performed as described by Vincent and Humphrey (1970).

Plant Inoculation

Effect of bacterial inoculation on plant growth was examined on glutinous corn since grasses are generally sensitive to nitrogen deficiency and responsive to supplemental N applications, through a pot experiment under screen-house condition. Seeds were planted on pots containing 1.5kg sterilized soil and were pre-germinated for four days with two seedlings per pot. Two days after germination, 5mL inoculum was applied at the base of the seedlings per pot. Six distinct bacterial isolates plus a negative control were used as treatments and set-up in Complete Randomized Design (CRD) with three replications per treatment. Plants were harvested 30 days after emergence and data sets were recorded for plant height, shoot and root dry weight, and total biomass.

Biochemical Characterization of Promising Isolates

Isolates LB1, LR1, and SC4 which showed plant growth-promoting activity were submitted to the Nueva Viscaya State University DNA-Based Indexing Laboratory for biochemical characterization using plate technique.

Statistical Analysis

Data on plant height, shoot biomass, root biomass, and total biomass were subjected to analysis of variance (ANOVA) and differences among treatment means were compared using least significant difference (LSD) test at 5% level using Statistical Tool for Agricultural Research (STAR) version 2.

Results and Discussion

Evaluation of Isolated Colonies

Population count of the 15 bacterial isolates ranged from 8×10^{7} to 4.8×10^{12} cfu g^{-1} of rhizosphere samples (Table 1). The high number of colonies observed from the

different samples implies greater microbial activities and higher possibility of PGPR isolation.

Table 2 presents the morphologic characteristics of the 15 bacterial isolates. Results indicate that 67% of the bacterial isolates were gram positive bacteria. Generally, most gram positive bacteria are beneficial whereas gram negative bacteria are pathogenic. All of the isolates were white to off-white, circular, with raised elevation, and had sharply-defined edges.

Screening for PGPR Activity on Corn Plants

Out of the 15 isolates subjected to morphological characterization, only six distinct isolates were evaluated for plant growth-promoting activity on corn plants. Table 3 shows that no significant differences on height were observed among treatments at 7 DAE. However, significant differences on height among the treated plants and the control were observed starting from 14 DAE to 28 DAE At 14 DAE, the height of corn plants inoculated with LR1 (34.90cm); LR3 (34.40cm), LR5, SR4 (34.37cm), and LB1 (33.70cm) was statistically the same, but SR5 (31.43cm) and the control (28.40cm) were significantly different. The relative increase in height of the inoculated plants over the control ranges from 7.15 – 22.89%. At 21 DAE, corn plants inoculated with LR5 (42.47cm), LR3 (40.93cm), and SR4 (39.30cm) were the tallest but comparable to those inoculated with SR5 (38.77cm), LB1 (37.43cm) and LR1 (37.23cm). The uninoculated corn plants were the shortest (34.03cm). At this stage, the relative increase in height of the inoculated plants over the control ranged from 9.40 to 24.80%. At 28 DAE, the same trend was observed except for LB1 (40.67cm), which was statistically the same as LR5 (44.50cm), SR5 (43.00cm), SR4 (42.50cm), and LR3 (42.33cm). The relative increase in height of the inoculated plants over the control ranged from 11.20 to 25.81%. Tiwari and Thrimurthy (2007) and Deepa et al (2010) suggests that PGPR can directly increase plant growth via different processes such as the following: atmospheric nitrogen fixation; siderophores production that chelates iron and makes it available to the plant roots; increased uptake of nitrogen, phosphorus, and potassium; and synthesis of phytohormones, indole acetic acid, gibberlic acid, and auxin.

Table 1. Population count of bacterial isolates from the rhizospheres and bulbs of sugarcane and wild lily

ISOLATE	POPULATION COUNT
Sugarcane	
SR1	8.0×10^{7}
SR2	1.7×10^{9}
SR3	7.0×10^{9}
SR4	5.7×10^{11}
SR5	4.0×10^{12}
Lily Bulb	
LB1	3.3×10^{8}
LB2	7.0×10^{8}
LB3	2.2×10^{10}
LB4	2.0×10^{10}
LB5	1.5×10^{12}
Lily Root	
LR1	4.9×10^{8}
LR2	2.5×10^{9}
LR3	2.7×10^{9}
LR4	5.3×10^{11}
LR5	4.8×10^{12}

On the other hand, significant differences were observed on the shoot biomass, root biomass, and total biomass of the test plants (Table 4). Corn plants inoculated with LB1 isolate produced the heaviest biomass (2.23g), which is 81.30% heavier than the control. Relative increase of shoot biomass of inoculated corn plants over the control ranged from 3.25 to 81.30%. In terms of root biomass, corn plants inoculated with LR1 obtained the heaviest with LR1 (1.03g), which was similar to those inoculated with LB1 (0.97g) and SC4 (0.87g) with relative increase of 74 – 106%. Others were comparable to the control, which ranged from 0.47 – 0.53g. For the

Table 2. Morphologic characteristics of selected bacterial isolates from the bulb and rhizospheres of wild lily and sugarcane

ISOLATE	GRAM REACTION	MORPHOLOGICAL CHARACTERISTIC
Sugarcane		
SR1	+	White, punctiform, raised colonies with entire margin
SR2	-	White, large circular, flat colonies with entire margin
SR3	+	Off-white, circular, flat colonies with entire margin
SR4	+	White, punctiform, raised colonies with entire margin
SR5	-	White, irregular, raised colonies with undulate margin
Lily Bulb		
LB1	+	Cream, filamentous, convex colonies with filiform margin
LB2	-	White, circular, flat colonies with entire margin
LB3	+	Off-white, punctiform, convex colonies with entire margin
LB4	-	White, circular, flat colonies with entire margin
LB5	+	White, irregular, convex colonies with undulate margin
Lily Root		
LR1	+	Off-white, circular, convex colonies with entire margin
LR2	-	White, circular, flat colonies with entire margin
LR3	+	White, irregular, flat colonies with undulate margin
LR4	+	White, filamentous, convex colonies with filamentous
LR5	+	White, irregular, flat colonies with entire margin

Table 3. Plant height (cm) of corn plants from 7 days to 28 DAE as affected by the bacterial isolates

TREATMENT	PLANT HEIGHT (cm)			
	7 DAE	14 DAE	21 DAE	28 DAE
	ns	**	*	*
Control	14.33	28.40 c	34.03 b	35.37 b
LR1	16.47	34.90 a	37.23 ab	39.33 ab
LR3	15.27	34.40 a	40.93 a	42.33 a
LR5	13.87	34.37 a	42.47 a	44.50 a
SR5	15.13	30.43 b	38.77 ab	43.00 a
SR4	16.77	34.37 a	39.30 a	42.50 a
LB1	14.37	33.70 ab	37.43 ab	40.67 a
CV (%)	8.63	5.61	7.00	6.64

In a column, means followed by the same letter are not significantly different using LSD at 5%.
CV – Coefficient of variations
ns – not significant * - significant at 5% level **- significant at 1% level

Table 4. Shoot, root and total biomass of corn plants at 28 DAE as affected by the bacterial isolates

TREATMENT	BIOMASS (g)		
	Shoot **	Root **	Total **
Control	1.23 c	0.50 b	1.74 d
LR1	1.53 bc	1.03 a	2.57 b
LR3	1.57 b	0.53 b	2.10 c
LR5	1.53 bc	0. 50 b	2.03 c
SC5	1.27 bc	0.47 b	1.73 d
SC4	1.53 bc	0.87 a	2.40 b
LB1	2.23 a	0.97 a	3.20 a
CV (%)	10.99	14.98	10.42

Means followed by the same letter in a column are not significantly different using LSD at 5%.
CV – Coefficient of variations
** - significant at 1% level

total biomass, corn plants inoculated with LB1 produced the heaviest with 3.20g that was significantly different from LR1 (2.57g) and SC4 (2.40g) that gave an increase over the control by 83.90%, 47.70% and 37.93%, respectively. Dey et al (2004) and Gray and Smith (2005) reported that rhizobacteria produce indole acetic acid, which is needed in root initiation, cell division, cell enlargement, and root surface area expansion, as well as, the consequent access to soil nutrients via enhanced root formation. Poonguzhali et al (2008) reported that the PGPR from the rhizosphere of *Brassica campestris* can produce 6.02–29.75 µg/ml of IAA. The variations in the ability of PGPR to produce IAA among plant growth-promoting bacteria were also reported earlier (Mansour et al, 1994; Zahir et al, 2000).The variations were due to the following: countless biosynthetic pathways; location of the genes involved; regulatory sequences; and the presence of enzymes to convert active free IAA into segmented forms, which are beneficial to the host plants (Patten and Glick, 1996).

Biochemical Characterization

Results of biochemical tests are presented in Table 5. They confirmed that the three promising bacterial isolates were gram positive. Their colony colors ranged from creamy white to dirty white. Nitrate reduction test was positive in all of the three isolates. This means that these bacteria are able to perform nitrification on nitrate and nitrite to produce molecular nitrogen. Reduction of either nitrate or nitrite provides energy for the growth of the bacteria in the absence of oxygen, with nitrate reduction to nitrite via nitrate reductase contributing more significantly to proton motive force (energy production), than nitrite reduction (Berks et al, 1995; Zumft, 1997).

Meanwhile, urease test showed negative reaction, which indicates that the bacteria cannot degrade urea via the enzyme urease.

Moreover, the citrate utilization test showed a positive reaction, which indicates that the bacteria can utilize citrate as their

sole carbon source. They use the enzyme citrase or citrate-permease to transport the citrate into the cell. These bacteria also convert the ammonium dihydrogen phosphate to ammonia and ammonium hydroxide, which creates an alkaline environment in the medium.

The three bacteria showed a positive reaction to the catalase test, which manifests that the bacteria are either aerobic or facultative anaerobes and could convert hydrogen peroxide into water and oxygen. Further, the starch hydrolysis test showed a positive reaction to the three bacteria, which means that the bacteria can produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase to degrade the starch into subunits for the organisms to utilize. On the other hand, sucrose and galactose tests showed negative reaction by the bacteria. However, Manitol tests showed positive reaction indicating that the bacteria are motile and can be used in the fermentation process. The positive reaction of the bacteria to xylose test indicates that they belong to the genus *Bacillus* (Sharma et al, 2013). Only isolate LB1 showed positive reaction to lactose, cellobiose, and fructose tests. Isolate LB1 can breakdown the cellobiose,

Table 5. Biochemical characteristics of the three promising plant-growth promoting bacteria

BIOCHEMICAL CHARACTERIZATION	**BACTERIAL ISOLATE**		
	LR1	**SR4**	**LB1**
Gram staining	+	+	+
Colony color	Creamy white	Creamy white	Dirty white
Nitrate reduction	+	+	+
Urease	+	+	+
Citrate utilization	+	+	+
Catalase	+	+	+
Acid processed from			
Sucrose	-	-	-
Galactose	-	-	-
Mannitol	+	+	+
Xylose	+	+	+
Lactose	-	-	+
Glucose	+	+	+
Mannose	+	+	+
Cellociose	-	-	+
Fructose	-	-	+
Maltose	+	+	+
Trehalose	+	+	+
Identification	*Bacillus subtiltis*	*Bacillus subtiltis*	*Paenibacillus polymyxa*

which is characterized by a disaccharide derived from the condensation of two glucose molecules linked in a bond. Based on the tests conducted, the promising isolates of LR1 and SR4 are *Bacillus subtilis,* as well as LB1, which is *Paenibacillus polymyxa*.

Reports revealed that Bacillus species showed yield increase in rice (Sudha et al, 1999), barley (Çakmakçı et al, 2001; Fahin et al, 2004), wheat (de Freitas, 2000; Çakmakçı et al, 2007), maize (Pal, 1998), sugar beet (Çakmakçı et al, 1999), and sugarcane (Sundara et al, 2002). Similarly, Bacillus strains increased the total bacteria and the PSB population, and/or root and shoot dry weight, as well as the total N and P uptake by plants (Canbolat et al, 2006). Plant growth responses were variable and dependent upon the inoculant strain, soil organic matter content, growing stage, harvest date, and growth parameters evaluated (Çakmakçı et al, 2006).

On the contrary, *Paenibacillus polymyxa* has a range of reported properties, including nitrogen fixation (Coelho et al, 2003; Çakmakçı et al, 2006), phosphorus solubilization (de Freitas et al, 1997), antibiotic production (Rosado and Seldin, 1993), cytokinin production (Timmusk et al, 1999), and increased root and shoot growth (Sudha et al, 1999).

Conclusion and Recommendation

The study successfully isolated, characterized, and evaluated the growth-promoting activity of bacterial isolates from the rhizospheres of sugarcane and wild lily. Among the six gram-positive bacterial isolates evaluated on the early growth stages of corn, LR1, SR4, and LB1 showed positive effects as revealed by the increased height (from 11.2-25.81%) of the inoculated plants at 28 DAE over the control. In addition, they increased the total biomass over the control by 37.93%, 47.70%, and 83.90%, respectively. Biochemical tests revealed that LR1 and SC4 are *Bacillus subtilis* and LB1 is *Paenibacillus polymyxa;* all of which are reported to have plant growth-promoting activity. However, molecular characterization should be done to confirm the identification of the promising bacterial isolates before they could be packaged as biofertilizers and be used in support to the organic agriculture program of the Department of Agriculture.

Acknowledgment

The authors are immensely indebted to the Philippine Council for Agriculture, Aquatic, and Natural Resources and Development (PCAARRD) for funding the Development of Organic Fertilizers and Microbials for Improved Soil Fertility in Organic Vegetable Production Enterprise in Region I project. The contribution of Dr. Jonar I. Yago of the Nueva Vizcaya State University in identifying the bacterial isolates and Prof. Beatriz S. Malab of the College of Agriculture, Food and Sustainable Development, Mariano Marcos State University in managing the operations of the project.

Literature Cited

Bais H.P., V.M. Loyola, H.E. Flores, and J.M. Vivanco. 2001. Root specific metabolism: the biology and biochemistry of underground organs. In vitro Cell Dev Biol Plant 37:730–741.

Berks, B.C., S.J. Ferguson, J.W.B. Moir, and D.J. Richardson. 1995 Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1232(3):97–173.

Botelho, G.R. and L.C. Mendonça-Hagler. 2006. Fluorescent P*seudomonads* associated with the rhizosphere of crops - an overview. Braz. J. Microbiol. 37:4

Breed, R. and W.D. Dotterrer. 1916. The Number of Colonies Allowable On

Satisfactory Agar Plates. J Bacteriol. 1:321-331.

Brimecombe, M.J., F.A.A.M. De Leij, and M.L. James. 2007. In: The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. *2nd edition* CRC Press. Tylor and Francis Grp. 73-109.

Çakmakçı, R. 2005a. Bitki gelifliminde fosfat çözücü bakterilerin önemi. Selçuk Üniv. Ziraat Fakültesi Dergisi 35: 93-108.

Çakmakçı, R. 2005b. Bitki geliflimini teflvik eden rizobakterilerin tarımda kullanımı. Atatürk Üniv. Ziraat Fakültesi Dergisi 36: 97107.

Çakmakçı, R., F. Dönmez, A. Aydın and F. Fahin. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol. Biochem. 38: 1482-1487.

Çakmakçı, R., F. Kantar and F. Fiahin. 2001. Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. J. Plant Nutr. Soil Sci. 164: 527-531.

Çakmakçı, R., F. Kantar and Ö.F. Algur. 1999. Sugar beet and barley yields in relation to Bacillus polymyxa and Bacillus megaterium var. phosphaticum inoculation. J. Plant Nutr. Soil Sci. 162: 437442.

Çakmakçı, R., M. Erat, Ü.G. Erdo¤an and M.F. Dönmez. 2007. The influence of PGPR on growth parameters, antioxidant and pentose phosphate oxidative cycle enzymes in wheat and spinach plants. J. Plant Nutr. Soil Sci. 170: 288-295.

Canbolat, M.Y., S. Bilen, R. Çakmakçı, F. Sahin, and A. Aydin. 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils. 42:350-357.

Coelho, M.R.R., I. Von der Weid, V. Zahner and L. Seldin. 2003. Character of nitrogen-fixing Paeni bacillus species by polymerase chain reaction-restriction fragment length polymorphism analysis o part of genes encoding 16S rRNA and 23S rRNA and by multilocus enzyme electrophoresis. FEMS Microbiol. Lett. 222: 243-250.

de Freitas, J.R. 2000. Yield and N assimilation of winter wheat (Triticum aestivum L., var Norstar) inoculated with rhizobacteria. Pedobiologia 44: 97-104.

de Freitas, J.R., M.R. Banerjee and J.J. Germida. 1997. Phosphatesolubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). Biol. Fertil. Soils 24: 358-364.

Deepa C. K., S.G. Dastager, and A. Pandey. 2010. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. *World J. Microbiol. Biotechnol.* 26 1233–1240.

Dey R., K.K. Pal, D.M. Bhatt, and S.M. Chauhan. 200). Growth promotion and yield enhancement of peanut (*Arachishypogaea* L.) by application of plant growth promoting rhizobacteria. *Microbiol. Res.* 159 371–394 10.1016/ j.micres.2004.08.004.

Estabrook E.M. and J.I Yoder. 1998. Plant-plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. Plant Physiol 116:1–7.

Flores H.E., J.M. Vivanco, and V.M. Loyola-Vargas. 1999. "Radicle" biochemistry: The biology of root-specific metabolism. Trends Plant Sci 4: 220–226

Giani, C., D. Wullbrandt, R. Rothert, and J. Meiwes. 1996. Pseudomonas aeruginosa and its use in a process for the biotechnological preparation of L-rhamnose.

Gray E.J. and D.L. Smith. 2005. Intracelluler and extracellular PGRP: commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem.* 37:395–412.

Gumtang, R.J., M.F. Pampolino, T.P. Tuong, and D.S. Bucao. 1999. Groundwater dynamics and quality under intensive cropping systems. Experimental Agriculture. 35(2):153-166.

Haque, N.A. and S.R. Dave. 2005. Ecology of phosphate solubilizers in semi-arid agricultural soils. Indian J Aiicrobiol., 45: 27-32.

Hardoim P.R., L.S. van Overbeek, and J.D. Elsas. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* 16 463–471 10.1016/j.tim.2008.07.008.

Holmes, A., J. Govan, and R. Goldstein. 1998. Agricultural Use of Burkholderia *(Pseudomonas)* cepacia: A Threat to Human Health?

Jansen, H.J. and Y. Bruinsma. 1989. Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to 15 N labeled ammonia. Soil Biol. Biochem. 21:189.

Kirk, J.L, L.A. Beaudette, H. Miranda, P. Moutoglis, J.N. Klironomos, H. Lee and J.T. Trevors. 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods. 58:169-188.

Kim YC, J. Leveau, B.B. McSpadden-Gardener, E.A. Pierson, L.S. Pierson III and C.M. Ryu. 2011. The multifactorial basis for plant health promotion by plant-associated bacteria. Appl. Environ. Microbiol. 77:1548–1555.

Lazarovits G. and J. Nowak. 1997. Rhizobacteria for improvement of plant growth and establishment. HortScience 32:188–192.

Mansour F.A., H.S. Ildesuguy, and H.A. Hamedo. 1994. Studies on plant growth regulators and enzyme production by some bacteria. *J. Qatar Univ. Sci.* 14 81–288.

Nardi S., G. Concheri, D. Pizzeghello, A. Sturaro, R. Rella, and G. Parvoli. 2000. Soil organic matter mobilization by root exudates. Chemosphere 5:653–658.

Ozdemir, G., N. Ceyhan, and E. Manav. 2005. Utilization of an exopolysaccharide produced by *Chryseomonas luteola* TEM05 in alginate beads for adsorption of cadmium and cobalt ions. Bioresource Technology (Vol. 96) (No. 15) 1677-1682.

Pal, S.S. 1998. Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 198: 169-177.

Patten C. and B.R. Glick. 1996. Bacterial biosenthesis of indole-3-acetic acid. *Can. J. Microbiol.* 42 207–220 10.1139/m96-032.

Persello-Cartieaux F., L. Nussaume and C. Robaglia. 2003. Tales from the underground: molecular plant-rhizobacterial interactions. Plant Cell Environ 26:189–199 Schroth MN and Hancock JG (1982) Disease-suppressive soil and root-colonizing bacteria. Science 216:1376-81.

Poonguzhali S., M. Madhaiyan M., and T.M. Sa. 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. *J. Microbiol. Biotechnol.* 18 773–777.

Rosado, A.S. and L. Seldin. 1993. Production of a potentially novel antimicrobial substance by Bacillus polymyxa. World J. Microbiol. Biotechnol. 9: 37-43.

Sharma, S.B., R.Z. Sayyed, M.H. Trivedi, and T.A. Gobi. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* 2: 587.

Shishido M, C. Breuil, and C.P. Chanway. 1999. Endophytic colonization of spruce by growth-promoting rhizobacteria. FEMS Microbiol. Ecol. 29:191–196.

Scialabba, N.E.H., and M. Müller-Lindenlauf. 2010. Organic agriculture and climate change. *Renewable Agriculture and Food Systems*, *25*(02), 158-169.

Singh, J. H. 2013. Plant Growth Promoting Rhizobacteria Potential Microbes for Sustainable Agriculture.

Sturtz, A.V., B.R. Cristie, and S. Nodwrwak. 2000. Bacterial endophytes potential role in developing sustainable system of crop production. Crit Rev Plant Sci 19:1-30.

Sudha, S.N., R. Jayakumar and V. Sekar. 1999. Introduction and expression of the cry1Ac gene of Bacillus thuringiensis in a cerealassociated bacterium, Bacillus polymyxa. Curr. Microbiol. 38: 163-167.

Sundara, B., V. Natarajan and K. Hari. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yield. Field Crop Res. 77: 43-49.

Tate, R. L. III. 2000. Soil Microbiology. *2nd edition*. John Wiley & Sons, Inc. 508pp. Vadakattu G. and Paterson, J. 2006. Free-living bacteria lift soil nitrogen supply. Farming Ahead. 169:40.

Tilman, D., K.G Cassman, P.A Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature, 418(6898), 671-677.

Timmusk, S., B. Nicander, U. Granhall, and E. Tillberg. 1999. Cytokinin production by Paenibacillus polymyxa. Soil Biol. Biochem. 31: 1847-1852.

Tiwari, P.K. and V.S. Thrimurthy. 2007. Isolation and characterization of the Pseudomonas fluorescens from rhizosphere of different crops. J Mycol Plant Pathol. 37:231–4.

Tomasiewicz, D.M. 1980. The Most Suitable Number of Colonies on Plates for Counting. J Food Prot. 43(4):282-286.

Vadakattu, G. and J. Paterson. 2006. Free-living bacteria lift soil nitrogen supply. Farming Ahead. 169:40.

Vessey, K.J. 2003. Plant growth promoting rhizobacteria as biofertilizer. Plant Soil 235:571-586.

Zahir A., S.A. Abbas, M. Khalid, and M. Arshad. 2000. Structure dependent microbially derived plant hormones by improving growth of maize seedlings. *Pak. J. Biol. Sci.* 3 289–291 10.3923/ pjbs.2000.289.291.

Zumft, WG. 1997. Cell biology and molecular basis of denitrification. See comment in PubMed Commons belowMicrobiol Mol Biol Rev. 61(4):533-616.