

**SEEDLING DEVELOPMENT AND GROWTH PROMOTION OF RICE (*Oryza Sativa*)
BY PLANT GROWTH PROMOTING BACTERIAL SEED TREATMENT**

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ABSTRACT

Plant growth promoting bacteria (PGPB) was used as bio fertilizer for different crop plants as an alternative source of chemical fertilizer that can reduce the input cost on farming. Two bacterial isolates, *Bacillus licheniformis* UPMC10 and *Acinetobacter* sp. JITUC7 were used in this study. Result from in vitro study showed that these bacteria were positive on siderophore and IAA production, phosphate solubilization and nitrogen fixation activity. Rice seeds treated *Bacillus licheniformis* UPMC10 recorded an increase of 14% seed germination compared to control. Similarly, an increase in vigor index of 659.00 was obtained when seeds were treated with *Acinetobacter* sp. JITUC7. Both bacterial isolates enhanced plant height, no. of tillers and grain weight when seeds were treated with fresh suspension of the bacteria. An average plant height of 128 cm was obtained when seeds were treated with *Bacillus licheniformis* UPMC10 and *Acinetobacter* sp. JITUC7. The average no. of tillers (13) and grain weight (120.67 g) were obtained when seeds were treated with *Bacillus licheniformis* UPMC10. *Bacillus licheniformis* UPMC10 is a potential PGPB evidenced by preliminary screening of plant growth promoting activities, in vitro and in vivo experiment. *Bacillus licheniformis* UPMC10 which able to adapt in environmental conditions and at the same time promote plant growth, has a wide potential to be developed into bio fertilizer..

Keywords: Plant growth promoting bacteria, seed treatment, rice, growth promotion

Introduction

Rice is one of the most important food crops in the world especially in the most of Asian countries. Rice is the staple food for more than half of the world population [1]. Demand for rice

has been growing due to population growth, rising incomes and shift in consumer preferences in favour of rice, especially in urban areas [2]. Sharp increases in food prices occurred in global and national markets in 2008, and the resulting increases in the number of hungry and malnourished people have sharpened the awareness of policy-makers and of the general public to the fragility of the global food system [3]. Among the food commodities concerned, it is the phenomenal rise in rice prices which has probably inflicted the greatest damage to food security of the poorest households in Asia [4].

Beneficial bacteria are often referred as plant growth promoting bacteria (PGPB). These beneficial bacteria are also referred as yield increasing bacteria [5]. PGPB are defined as free-living soil, rhizosphere, rhizoplane and phyllosphere bacteria that, under some conditions, are beneficial for plants [6]. PGPB encompasses all bacteria that exert positive effect on plant development by various mechanisms [7].

PGPB strains use one or more direct or indirect mechanisms to enhance the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth [7]. PGPB have been reported to directly enhance plant growth by a variety of mechanisms such as fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones like indole-3-acetic acid (IAA), gibberellic acid, cytokinins, and ethylene [8]. Indirect mechanisms involve the biological control of plant pathogens and deleterious microbes [9], through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and siderophore or through competition for nutrients and space [10].

PGPB have been demonstrate to increase growth and productivity of many commercial crops including rice [11]-[14], wheat [15]-[20], cucumber [21], maize [22], [23], cotton [24], black pepper [25], banana [26], tomato [27], lettuce [28], soybean [29], groundnut [30], broad bean [31], chickpea [32], barley [33]-[35], sugar beet [36], strawberry [37], apple [38], grapes [39] and raspberry [40].

Plant growth promoting strains of *Bacillus* have been widely studied for enhancement of plant growth [41], [42]. *Bacillus* had plant growth promoting traits such as IAA production, phosphate solubilization, nitrogen fixation and siderophore production [43]-[46]. *Acinetobacter* sp. isolates were confirmed to be PGPB by their capabilities for nitrogen fixation, IAA production, phosphate solubilization and siderophore production [47], [48].

The aim of this study was to evaluate seedling development and growth promotion of rice (*Oryza sativa*) by plant growth promoting bacterial seed treatment.

2. Material and Methods

2.1 Bacterial and Inoculum Preparation

Two bacterial isolates, *Bacillus licheniformis* UPMC10 (KT958890.1) and *Acinetobacter* sp. JITUC7 (KT958891.1) was isolated and characterized by molecular method prior to this study. Bacterial cell suspensions were prepared by growth in nutrient broth for 24 hours. After 24 hours, the bacterial cells were harvested by centrifugation at 8000 rpm for 5 min. The pellets obtained were suspended in sterile distilled water and again subjected to centrifugation, and the supernatants were discarded. The pellets were finally collected in sterile distilled water and cell populations were adjusted to 10⁸ CFU/ml as measured spectrophotometrically [49].

2.2 Screening of Plant Growth Promoting Activity

2.2.1 Indole Acetic Acid (IAA) Production

Bacterial isolate were grown in NB and incubated in incubator shaker with 150 rpm agitation at room temperature (28 ± 2°C) for 24 hours. One ml of bacterial culture was inoculated into 100 ml of sterile NB amended with 5 ml L-TRP solution and grows for 48 hours. The broth was centrifuged at 12000 rpm for 5 minutes and 1 ml of supernatant was added to 2 ml Salkowski reagent. The colour density of the mixture (red colour development which indicated IAA production) was measured using UV spectrophotometer at 530 nm absorbance [50]. The amount of IAA production was determined based on standard curve of IAA. The experiment was performed with a completely randomized design with three replications.

2.2.2 Siderophore Production

Bacterial suspension (10 µl) was dispensed by using pipette onto sterile filter paper (6.00 mm) which was placed on Chrome azurol S (CAS) agar [51] and incubated at 30°C for 5 days. Development of yellow-orange halo zone around the bacterial growth was considered as positive for siderophore production and the diameter were measured. Experiment was performed with a completely randomized design with three replications.

2.2.3 Phosphate Solubilization

Bacterial suspension (10 µl) was dispensed by using pipette onto sterile filter paper (6.00 mm) which was placed on National Botanical Research Institute's phosphate growth medium (NBRIP) [52] and incubated at 28°C for 7 days. Phosphate solubilization activity was assessed by measuring the clear halo zone around bacterial colony. The experiment was performed with a completely randomized design with three replications.

2.2.4 Nitrogen Fixation

Bacterial suspension (10 µl) was dispensed by using pipette onto sterile filter paper (6.00 mm) which was placed on nitrogen free media [53]. After 7 days of incubation period at 28±2°C, the

isolates ability to fix nitrogen was observed by green to blue coloration of the nitrogen free media. The experiment was performed with a completely randomized design with three replications.

2.3 Effect of PGPB on Seed Development and Growth Promotion of Rice

2.3.1 Rice Seed Treatments

Bacillus licheniformis UPMC10, *Acinetobacter* sp. JITUC7 and their combination were used as fresh suspension. Seed of MR219 obtained from Malaysian Agriculture Research and Development Institute (MARDI). Seed of MR219 were surface sterilized with 2% sodium hypochlorite for two minutes. Seed treatments was achieved by soaking rice seeds in 108 CFU/ml bacterial suspension (100 g/500 ml), prepared as described earlier, and amended with 0.2% sterilized carboxymethyl cellulose (CMC) as a sticker. After that, seeds that treated with bacterial suspension were incubated at 26°C in incubator shaker at 150 rpm for 6 hours to facilitate attachment of bacterial cells to the seed coat. Later, the seed were allowed to dry in incubator at 30°C. The seeds treated with sterile distilled water amended with CMC served as controls.

2.3.2 In vitro/Laboratory Assessment

The germination tests were carried out according to the paper towel method. PGPB treated seeds and control were seeded onto paper towels. One hundred seeds were placed equally on the germination paper pre-soaked in distilled water and covered with another pre-soaked paper towel. Arranged seeds on paper towel were rolled up along with polythene wrapping to prevent drying of towels. The rolled towels were incubated for 14 days at 24±1°C. After incubation, paper towels were unrolled and number of germinated seeds were counted and represented in percentage. The vigor index was calculated by using the formula [54]. To assess vigor, the length of the root and shoot of an individual seedling was measured. The vigor Index was calculated using the formula $VI = (\text{mean root length} + \text{mean shoot length}) / \text{germination} (\%)$. The experiment was performed with a completely randomized design with three replications.

2.3.3 In vivo/Glasshouse Assessment

A glasshouse experiment was conducted at Ladang 2 complex of Univeristi Putra Malaysia (UPM). Seeds treated with PGPB and control was prepared as described earlier. Soil obtained from rice fields in Tanjung Karang, Selangor was sterilized at 240V for 1.5 hours. Fifteen kg of sterile paddy soil were filled in plastic pails and all were applied nitrogen (2.1g Urea pot⁻¹), phosphorus (1.2g P₂O₅ pot⁻¹) and potassium (1.1g K₂O pot⁻¹) fertilizers according to MARDI recommended rates NPK 120:70:80 kg/ha. Fresh tap water was the main water source used in

the glasshouse experiment. During the growth of seedlings establishment, the water level was maintained at 4 cm above the soil surface. The flooding was maintained throughout the growth period until two weeks before harvesting when the water was drained out in order to hasten ripening and drying of the grains. During the experimental period, the occurrence of other pests and diseases were closely monitored in order to avoid yield losses due to other factors including insects and weeds. Rice plants were cover by net in order to avoid insect's infestation. Plant height, no. of tillers and grains weight were measured after 120 days of planting. The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replication of three seeds each plastic vials.

3. Results

3.1 Screening of Plant Growth Promoting Activity

Bacillus licheniformis UPMC10 and *Acinetobacter* sp. JITUC7 were able to produce IAA at 4.42 and 4.16 mg/L respectively. Both of these bacterial were also able to produce siderophore shown by 6.00 and 10.00 mm orange halo zone around bacteria colony on CAS agar respectively. These two strains were able to solubilize phosphate indicated by 11.00 and 12.33 mm clear halo zone around the bacteria growth on NBRIP agar respectively. Both of these bacterial strains also showed positive result on nitrogen fixation activity evidenced by green to blue coloration of the nitrogen free media (Table 1).

Table 1: Preliminary screening of plant growth promoting activities

Bacterial strains	Siderophore production (mm)	IAA production (mg/L)	Phosphate solubilization (mm)	Nitrogen fixation
<i>Bacillus licheniformis</i> UPMC10	6.00 ± 0.58 ^b	4.42 ± 0.26 ^a	11.00 ± 0.00 ^a	Positive
<i>Acinetobacter</i> sp. JITUC7	10.00 ± 0.58 ^a	4.16 ± 0.05 ^a	12.33 ± 0.88 ^a	Positive

* Means in column followed with same letter (s) are not significantly different (t-test P>0.05)

3.2 Effect of PGPB on Seed Development and Growth Promotion of Rice

3.2.1 In vitro/Laboratory Assessment

There was significant different on percentage germination, shoots length, roots length, and vigor index of seedlings for treatment with PGPB compared with control. The percentage germination of rice seeds treated with different PGPB ranged from 86.67% to 89.33%. Percent germination of control seeds without PGPB treatment was 75.33%. The shoots length of seedlings with PGPB treatment ranged from 7.54 cm to 8.47 cm, whereas for control seedlings, the shoots length was 7.48 cm. The roots length of seedlings ranged from 11.19 cm to 12.74 cm for seeds treated with PGPB, compared with 8.72 cm in the control. The vigor index of rice seedlings treated with different PGPB ranged from 1625.30 to 1874.60. The vigor index of control seedlings without PGPB treatment was 1220.60 (Table 2).



Figure 1: In vitro assessment on seed germination and seed vigor performance with *Bacillus licheniformis* UPMC10 and *Acinetobacter* sp. JITUC7 using roll towel method (14 days assessment)

Table 2: Effect of seed treatment with PGPB on seed germination and seedling vigor of rice under laboratory conditions

Treatment	Germination (%)	Shoots length (cm)	Roots length (cm)	Vigor Index
<i>Bacillus licheniformis</i> UPMC10	89.33 ± 3.38 ^a	8.00 ± 0.22 ^{ab}	12.32 ± 0.15 ^a	1816.70 ± 88.05 ^a
<i>Acinetobacter</i> sp. JITUC7	89.06 ± 2.65 ^a	8.47 ± 0.23 ^a	12.74 ± 1.05 ^a	1874.60 ± 184.4 ^a
<i>Bacillus licheniformis</i> UPMC10 + <i>Acinetobacter</i> sp. JITUC7	86.67 ± 1.20 ^a	7.54 ± 0.14 ^b	11.19 ± 1.02 ^{ab}	1625.30 ± 117.27 ^{ab}
Control	75.33 ± 1.45 ^b	7.48 ± 0.18 ^b	8.72 ± 0.08 ^b	1220.60 ± 42.42 ^b

* Means in column followed with same letter (s) are not significantly different (Tukey's Test P>0.05)

3.2.2 In vivo/Glasshouse Assessment

There was significant different on plant height, no. of tillers and grain weight of plant treated with PGPB compared with control. In general, all the PGPB treated plants showed positive growth responses among all parameters recorded under glass house conditions compared with the control. Specifically all the PGPB enhanced plant height, no. of tillers and grain weight. The highest plant height of 128 cm resulted from plants treated with *Bacillus licheniformis* UPMC10 and *Acinetobacter* sp. JITUC7. The highest no. of tillers of 13 and grain weight of 120.67 g was recorded by plants treated with *Bacillus licheniformis* UPMC10 (Table 3).

Table 3: Effect of seed treatment with PGPB on growth promotion of rice under glasshouse conditions

Treatment	Plant height (cm)	Tillers/plant	Grain weight/pot (g)
<i>Bacillus licheniformis</i> UPMC10	128 ± 2.08 ^a	13 ± 0.33 ^a	120.67 ± 3.07 ^a
<i>Acinetobacter</i> sp. JITUC7	128 ± 2.08 ^a	12 ± 0.57 ^a	113.20 ± 6.86 ^a
<i>Bacillus licheniformis</i> UPMC10 + <i>Acinetobacter</i> sp. JITUC7	127 ± 0.33 ^a	12 ± 0.33 ^a	113.45 ± 2.69 ^a
CONTROL	121 ± 0.67 ^b	10 ± 0.33 ^b	81.6 ± 1.31 ^b

Means in column followed with same letter (s) are not significantly different (Tukey's Test $P > 0.05$)

4. Discussion

4.1 Screening of Plant Growth Promoting Activity

From in vitro screening, both bacteria demonstrated a promising potential in plant growth promotion. Ability to produce siderophore and IAA are essential for plant growth promoter. *Bacillus* spp. and *Acinetobacter* spp. have been reported and well known for siderophore and IAA production by numerous studies [26], [47], [55]-[61]. Bacteria with phosphorus solubilization and nitrogen fixation ability may provide an available form of phosphorus and nitrogen for plant growth development. Utilization of plant growth promoter with phosphate solubilizing characteristics could reduce phosphate fertilizer application by 50% [62], [63]. Kennedy et al., (2004) [64] stated in their study that nitrogen-fixing bacterial can fix annually great amounts of nitrogen which equivalent to 60-90kg N ha⁻¹. Therefore, utilization of nitrogen-fixing bacteria is cost wise and environmental friendly compared to conventional nitrogen fertilizer. *Bacillus* was among the most powerful phosphate-solubilizing microorganism [65] which abundantly populated in soil ecosystem.

4.2 Effect of PGPB on Seed Development and Growth Promotion of Rice

4.2.1 In vitro/Laboratory Assessment

Based on preliminary screening in this experiment, treated seeds demonstrated higher value ($p > 0.05$) compared to control treatment in each parameter examined in this study (germination percentage, shoot length, root length and vigor index). A number of studies suggest that PGPB enhances seed emergence, plant growth, crop yield, and contribute to the protection of plants against certain pathogens and pests [41], [66]-[69]. PGPB inoculation significantly enhanced seed germination and seedling vigor, however, the rate of enhancement varied with bacterial strains [70].

4.2.2 In vivo/Glasshouse Assessment

Result trend for rice growth promotion as shown in Table 3 was similar to seed germination and seedling vigor experiment, where treated seeds demonstrated higher result ($p > 0.05$) compared to untreated seeds. Although there was no significant different among treated seeds ($p > 0.05$), single treatment of *Bacillus licheniformis* UPMC10 demonstrated higher grain weight and tiller production compared to single treatment of *Acinetobacter* sp. JITUC7 and combination of *Bacillus licheniformis* UPMC10 and *Acinetobacter* sp. JITUC7. Although *Acinetobacter* sp. JITUC7 demonstrated promising result during preliminary screening and seedling germination, *Bacillus licheniformis* UPMC10 showed higher value in grain weight and tiller production per plant. This is due to the ability of *Bacillus* to survive in in vivo experiment where environmental factors could be the constraint.

The variability in the performance of PGPB may be due to various environment factors that may affect their growth and exert their effects on plant. The environmental factors include climate, weather conditions, and soil characteristics of the composition or activity of the indigenous microbial flora of the soil [70]. Factors affecting the survival of bacterial in the soil include certain abiotic factors including soil temperature and moisture, nutrient presence and pH of the soil [71]. Efficient colonization also supports better functioning of PGPB [72]. PGPB also have to compete with the local bacteria and other soil organism in the root zone for colonization [73], [74]. In this study, *Bacillus licheniformis* UPMC10 ascertain its potential as a good candidate of PGPB through in vivo experiment due to several factors. Nelson (2004) [8] mentioned that *Bacillus* survive for prolonged periods in competitive and challenged environments. Garcia et al., (2004) [75] proved that inoculation of *Bacillus licheniformis* on tomato and pepper demonstrated considerable colonization which can be used as biofertilizer without altering normal management in greenhouse.

5. Conclusion

Our results demonstrate that *Bacillus licheniformis* UPMC10 has a potential as plant growth promoter, which is an added advantage for practical agriculture system. It is evidence that these bacteria could possibly serve as eco-friendly and sustainable alternative to the hazardous chemicals used for management of crop disease and growth promotion.

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References

- [1] M.A. Akhtar, A. Rafi, A. Hameed, "Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivar", *Pakistan Journal of Botany*, 40, pp. 2171-2175, 2008.
- [2] E.A. Somado, R.G. Guei, S.O. Keya, *NERICA: the new rice for Africa - a Compenium*. Cotonou, Benin: Africa Rice Center (WARDA); Rome, Italy: FAO; Tokyo, Japan: Sasakawa Africa Association, pp. 210, 2008.
- [3] FAO, "How to Feed the World in 2050", Rome, Italy, p. 35, 2009. [Online]. Available: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf. [Accessed: May 25, 2016]
- [4] P.A. Seck, E. Tollens, M.C.S. Wopereis, A. Diagne, I. Bamba, "Rising trends and variability of rice prices. Threats and opportunities for Sub-Saharan Africa", *Food Policy*, 35(5), pp. 403-411, 2010.
- [5] A.O. Adesemoye, H.A. Torbert, J.W. Kloepper, "Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system", *Canadian Journal of Microbiology*, 54, pp. 876-886, 2008.
- [6] Y. Bashan, L.E. de-Bashan, "Plant Growth-Promoting", in *Encyclopedia of soils in the environment*, D. Hillel (ed.), Elsevier, Oxford, U.K, pp. 103-115, 2005.
- [7] F. Ahmad, I. Ahmad, M.S. Khan, "Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities", *Microbiological Research*, 163, pp. 173-181, 2008.

- [8] L.M. Nelson, "Plant growth promoting rhizoacteria (PGPR): prospects for new inoculants", *Online Crop Management*, doi, 10.1094/CM-2004-0301-05-RV, 2004.
- [9] B.R. Glick, "Plant growth-promoting bacteria: mechanism and application", *Scientifica*, doi, 10.6066/2012/963401, 2012.
- [10] M.S. Khan, A. Zaidi, P.A. Wani, "Role of phosphate-solubiliizng microorganism in sustainable agriculture- a review", *Agronomy for Sustainable Development*, 27, pp. 29-43, 2006.
- [11] M. Ashrafuzzaman, F.A. Hossen, M.R Ismail, M.A. Hoque, M.Z. Islam, S.M. Sahidullah, S. Meon, "Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth", *African Journal of Biotechnology*, 8, pp. 1247-1252, 2009.
- [12] H.B. Bal, L. Nayak, S. Das, T.K. Adhya, "Isolation of ACC deaminase PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress", *Plant and Soil*, 366, pp. 93-105, 2013.
- [13] A. Jha, J. Saxena, V. Sharma, "An investigation on phosphate solubilisation potential of agricultural soil bacteria as affected by different phosphorus sources, temperature, salt and pH", *Communications in Soil Science and Plant Analysis*, 44, pp. 2443-2458, 2013.
- [14] Lavakush, J. Yadav, J.P. Verma, D.K. Jaiswal, A. Kumar, "Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*)", *Ecological Engineering.*, 62, pp. 123-128, 2014.
- [15] A. Khalid, M. Arshad, Z.A. Zahir, "Screening plant growth promoting rhizobacteria for improving growth and yield of wheat", *Journal of Applied Microbiology*, 96, pp. 473-480, 2004.
- [16] L. Jaderlund, V. Arthurson, U. Granhall, J.K. Jansson, "Specific interactions between arbuscular mycorrhizal fungi and plant growth promoting bacteria: as revealed by different combinations", *FEMS Microbiology Letters*, 287, pp. 174-180, 2008.
- [17] U. Chakraborty, B.N. Chakraborty, A.P. Chakraborty, P.L. Dey, "Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria", *World Journal of Microbiology and Biotechnology*, 29, pp. 789-803, 2013.
- [18] S.M. Nadeem, Z.A. Zaheer, M. Naveed, S. Nawaz, "Mitigation of salinity induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions", *Annals of Microbiology*, 63, pp. 225-232, 2013.

- [19] F. Islam, T. Yasmeen, Q. Ali, H. Rizvi, "Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress", *Ecotoxicolog and Environmental Safety*, 104, pp. 285-293, 2014.
- [20] A. Kumar, B.R. Maurya, R. Raghuwanshi, "Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.)", *Biocatalysis and Agricultural Biotechnolog*, 3, pp. 121-128, 2014.
- [21] M. Maleki, S. Mostafee, L. Mokhaternejad, M. Farzaneh, "Characterization of *Pseudomonas fluorescens* strain CV6 isolated from cucumber rhizosphere in Varamin as a potential biocontrol agent", *Australian Journal of Crop Science*, 4, pp. 676-683, 2010.
- [22] V. Sandhya, S.K.Z. Ali, M. Grover, G. Reddy, B. Venkatswarlu, "Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress", *Plant Growth Regulation*, 62, pp. 21-30, 2010.
- [23] D. Rojas-Tapias, A. Moreno-Galvan, S. Pardo-Díaz, M. Obando, D. Rivera, R. Bonilla, "Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*)", *Applied Soil Ecology*, 61, pp. 264-272, 2012.
- [24] M.A. Anjum, M.R. Sajjad, N. Akhtar, M.A. Qureshi, A. Iqbal, A. Rehman, Mahmud-ul-Hasan, "Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen", *Journal of Agricultural Research*, 45, pp. 135-143, 2007.
- [25] S.G. Dastager, C.K. Deepa, A. Pandey, "Potential plant growth promoting activity of *Serratia nematophila* NII-0.928 on black pepper (*Piper nigrum* L.)", *World Journal of Microbiolog and Biotechnology*, 27, pp. 259-265, 2010.
- [26] M.A.B. Mia, Z.H. Shamsuddin, Z. Wahab, M. Marziah, "Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured *Musa* plantlets under nitrogen-free hydroponics condition", *Australian Journal of Crop Science*, 4, pp. 85-90, 2010.
- [27] O.A. Almaghrabi, S.I. Massoud, T.S. Abdelmoneim, "Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions", *Saudi Journal of Biological Sciences*, 20, pp. 57-61, 2013.

- [28] Kohler, J.A. Hernandez, F. Caravaca, A. Roldan, "Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress", *Environmental and Experimental Botany*, 65, pp. 245-252, 2009.
- [29] O. Masciarelli, A. Llanes, V. Luna, "A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation", *Microbiological Research*, 169, pp. 609-615, 2014.
- [30] N.S. Paulucci, L.A. Gallarato, Y.B. Reguera, J.C. Vicario, A.B. Cesari, G. de Lema, M.S. Dardanelli, "*Arachis hypogaea* PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity", *Microbiological Research*, 173, pp. 1-9, 2015.
- [31] O. Younesi, A. Moradi, "Effects of plant growth-promoting rhizobacterium (PGPR) and arbuscular mycorrhizal fungus (AMF) on antioxidant enzyme activities in salt-stressed bean (*Phaseolus vulgaris* L.)", *Agriculture (Pol'nohospodárstvo)*, 60(1), pp. 10-21, 2014.
- [32] H.A. Patel, R.K. Patel, S.M. Khristi, K. Parikh, G. Rajendran, "Isolation and characterization of bacterial endophytes from *Lycopersicon esculentum* plant and their plant growth promoting characteristics", *Nepal Journal of Biotechnology*, 2, pp. 37-52, 2012.
- [33] A. Ozturk, O. Caglar, F. Sahin, "Yield response of wheat and Barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilizers", *Journal of Plant Nutrition and Soil Science*, 166, pp. 262-266, 2003.
- [34] A. Salantur, A. Ozturk, S. Akten, F. Sahin, F. Donmez, "Effect of inoculation with non-indigenous and indigenous rhizobacteria of Erzurum (Turkey) origin on growth and yield of spring barley", *Plant and Soil*, 275, pp. 147-156, 2005.
- [35] M.M.G. Turan, R. Cakmak, F. Sahin, "Effect of plant growth promoting rhizobacteria strain on freezing injury and antioxidant enzyme activity of wheat and barley", *Journal of Plant Nutrition*, 36, pp. 731-748, 2013.
- [36] F. Sahin, R. Cakmakci, F. Kantar, "Sugar beet and barley yields in relation to inoculation with N₂-fixing and phosphate solubilizing bacteria", *Plant and Soil*, 265, pp. 123-129, 2004.
- [37] A. Esitken, H.E. Yildiz, S. Ercisli, M.F. Donmez M. Turan, A. Gunes, "Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry". *Scientia Horticulturae*, 124, pp. 62-66, 2010.

- [38] R. Aslantas, R. Cakmakci, F. Sahin, "Effect of plant growth promoting rhizobacteria on young apples trees growth and fruit yield under orchard conditions", *Scientia Horticulturae*, 111, pp. 371-377, 2007.
- [39] C. Kose, M. Guleryuz, F. Sahin, I. Demirtas, "Effects of some plant growth promoting rhizobacteria (PGPR) on graft union of grapevine". *Journal of Sustainable Agriculture*, 26, pp. 139-147, 2005.
- [40] E. Orhan, A. Esitken, S. Ercisli, M. Turan, F. Sahin, "Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry", *Scientia Horticulturae*, 111(1), pp. 38-43, 2006.
- [41] J.W. Kloepper, C-M. Ryu, S. Zhang, "Induced systemic resistance and promotion of plant growth by *Bacillus* spp.", *Phytopathology*, 94, pp. 1259-1266, 2004.
- [42] D.K. Choudhary, B.N. Johri, "Interactions of *Bacillus* spp. and plants - with special reference to induced systemic resistance (ISR)", *Microbiological Research*, 164, pp. 493-513, 2008.
- [43] N. Raddadi, A. Cherif, A. Boudabous, D. Doffanchio, "Screening of plant growth promoting traits of *Bacillus thuringiensis*", *Annals of Microbiology*, 58, pp. 47-52, 2008.
- [44] P. Trivedi, A. Pandey, "Plant growth promotion abilities and formulation of *Bacillus megaterium* strain B 388 (MTCC 6521) isolated from a temperate Himalayan location", *Indian Journal of Microbiology*, 48, pp. 342-347, 2008.
- [45] M. Senthikumar, K. Swarnlakshmi, V. Govindasamy, Y.K. Lee, K. Annapurna, "Biocontrol potential of soybean bacterial endophytes against charcoal rot fungus *Rhizoctonia bataticola*", *Current Microbiology*, 58, pp. 288-293, 2009.
- [46] M. Maleki, S. Mostafee, L. Mokhaternejad, M. Farzaneh, "Characterization of *Pseudomonas fluorescens* strain CV6 isolated from cucumber rhizosphere in Varamin as a potential biocontrol agent", *Australian Journal of Crop Science*, 4, pp. 676-683, 2010.
- [47] D.V. Maindad, V.M. Kasture, H. Chaudhari, D.D. Dhavale, B.A. Chopade, D.P. Sachev, "Characterization and fungal inhibition activity of siderophore from wheat rhizosphere associated *Acinetobacter calcoaceticus* strain", *HIRFA* 32, 54(3), pp. 315-322, 2014.
- [48] J.C. Martínez-Rodríguez, M.D. Mora-Amutio, L.A. Plascencia-Correa, E. Audelo-Regalado, F.R. Guardado, E. Hernández-Sánchez, Y.J. Peña-Ramírez, A. Escalante, M.J. Beltrán-García, T. Ogura, "Cultivable endophytic bacteria from leaf bases of *Agave tequilana*"

and their role as plant growth promoters”, *Brazilian Journal of Microbiology*, 45(4), pp. 1333-1339, 2014.

[49] D.C. Thompson, “Evaluations of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass”, *Plant Disease*, 80, pp. 856-862, 1996.

[50] C.L Patten, B.R. Glick, “Bacterial biosynthesis of indole-3- acetic acid”, *Canadian Journal of Microbiology*, 42, pp. 207-220, 1996.

[51] D.B. Alenxander, D.A. Zuberer, “Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria”, *Biology and Fertililty of Soils*, 12, pp. 39-45, 1991.

[52] C.S. Nautiyal, “An afficient microbiological growth medium for screening phosphate solubilising microorganisms”, *FEMS Microbiology Letters*, 170, pp. 265-270, 1999.

[53] D.C. Eskew, D.D. Focht, I.P. Ting, “Nitrogen fixation, denitrification and pleomorphic growth in a highly pigmented *Spirillum lipoferum*”, *Applied and Environmental Microbiology*, 34, pp. 582-585, 1977.

[54] A.A. Abdul-Baki, J.D. Anderson, “Vigor determination in soybean seed by multiple criteria”, *Crop Science*, 13, pp. 630-633, 1973.

[55] D. Sachdev, V. Agarwal, P. Verma, Y. Shouche, P. Dhakephalkar, B. Chopade. “Assessment of microbial biota associated with rhizosphere of wheat (*Triticum aestivum*) during flowering stage and their plant growth promoting traits”, *The Internet Journal of Microbiology*, 7(2), 2008.

[56]A. Gulati, P. Vyas, P. Rahi, R.C. Kasana, “Plant growth-promoting and rhizosphere-competent *Acinetobacter rhizosphaerae* strain BIHB 723 from the cold deserts of the Himalayas”, *Current Microbiology*, 58, pp. 371-377, 2009.

[57] J.J. Acuna, M.A. Jorquera, O.A. Martinez, D. Menezes-Blackburn, M.T. Fernandez, P. Marschnev, R. Greiner, M.L Mora, “Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals”, *Journal of Soil Science and Plant Nutrition*, 11(3), pp. 1-12, 2011.

[58] R-Z. Farokh, D. Sachdev, N. Kazemi-Pour, A. Engineer, K.R. Pardesi, S. Zinjarde, P.K. Dhakephalkar, B.A. Chopade, “Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*’, *Journal of Microbiolog and Biotechnology*, 21(6), pp. 556-566, 2011.

- [59] B. Mohite, "Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth", *Journal of Soil Science and Plant Nutriiton*, 13(3), pp. 638-649, 2013.
- [60] Y. Ma, R.S. Oliveira, L. Wu, Y. Luo, M. Rajkumar, "Inoculation with metal-mobilizing plant growth-promoting rhizobacteria *Bacillus* sp. SC2b and its role in rhizoremediation", *Journal of Toxicology and Environmental Health*, 78, pp. 931-944, 2015.
- [61] J. Shim, J-W. Kim, P.J Shea, B-T. Oh, "IAA production by *Bacillus* sp. JH 2-2 promotes Indian mustard growth in the presence of hexavalent chromium", *Journal of Basic Microbiology*, 55, pp. 652-658, 2015.
- [62] G. Jilani, A. Akram, R.M. Ali, F.Y. Hafeez, I.H. Shamsi, A.N. Chaundhry, A.G. Chaundhry, "Enhancing crop growth, nutrient availability, economics and beneficial rhizosphere microflora through organic and biofertilizer", *Annals of Microbiology*, 57, pp. 177-183, 2007.
- [63] M. Yazdani, M.A. Bahmanyar, H. Pirdashti, M.A. Esmaili, "Effect of phosphate solubilisation microorganism (PSM) and plant growth promoting rhizobacteaia (PGPR) on yield and yield component of corn (*Zea mays* L.)", *World Academy of Science, Engineering and Technology*, 37, pp. 90-92, 2009.
- [64] I.R. Kennedy, A.T. Choudhury, M.L. Keeskes, "Non-symbiotic bacterial diazotrophs in crop-farming system: can their potential for plant growth promoting be better exploited", *Soil Biology and Biochemistry*, 3, pp. 1229-1244, 2004.
- [65] A. Fioretto, A. Fugggi, "Biotechnology of soil enzyme", in *Microbiol Biotechnology in Agriculture and Aquaculture*, Soil Science, R.C. Ray (ed.), New Hamsphire, pp. 31-70, 2005.
- [66] N. Kokalis-Burelle, J.W. Kloepper, M.S. Reddy, "Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms", *Applied Soil Ecology*, 31, pp. 91-100, 2006.
- [67] M.A.B. Herman, B.A. Nault, C.D. Smart, "Effects of plant growth promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York", *Crop Protection*, 27, pp. 996-1002, 2008.
- [68] M. Abdulkareem, H.M. Aboud, H.M., Saoud, M.K Shibly, "Antagonistic activity of some plant growth promoting rhizobacteria to *Fusarium graminearum*", *International Journal of Phytopathology*, 03(01), pp. 49-54, 2014.

[69] S. Hassen, G. Chellappan, V. Rethinasamy, “Efficacy of *Bacillus subtilis* G-1 in suppression of stem rot caused by *Sclerotium rolfsii* and growth promotion of groundnut”, *International Journal of Agriculture Environment and Biotechnology*, 8, pp. 111-118, 2015.

[70] D. Dasgupta, A. Ghata, A. Sarkar, C. Sengupta, G. Pool, “Application of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere *Sesbania bispinosa* on the growth of Chickpea (*Cicer arietinum* L.)”, *International Journal of Current Microbiology and Applied Science*, 4(5), pp. 1033-1042, 2015.

[71] R. Garcia, J. Balum, L. Fredslund, P. Santorum, C.S. Jacobsen, “Influence of temperature and predation on survival of *Salmonella enterica* Serovar Typhimurium and expression of *invA* in soil and manure-amended soil”, *Applied and Environmental Microbiology*, 76, pp. 5025-5031, 2010.

[72] S. Compant, C. Clement. A. Sessitsch, “Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization”, *Soil Biology and Biochemistry*, 42, pp. 669-678, 2010.

[73] J.K. Vessey, “Plant growth promoting rhizobacteria as biofertilizers”, *Plant and Soil*, 255, pp. 571-586, 2003.

[74] T.S. Walker, H.P. Bais, E. Grotewold, M. Vivanco, “Root exudation and rhizosphere biology”, *Plant Physiology*, 132, pp. 44-51, 2003.

[75] R. Garcia, J. Balum, L. Fredslund, P. Santorum, C.S. Jacobsen, “Influence of temperature and predation on survival of *Salmonella enterica* Serovar Typhimurium and expression of *invA* in soil and manure-amended soil”, *Applied and Environmental Microbiology*, 76, pp. 5025-5031, 2010.