

Effect of Rhizobacterial Isolates on Sweet potato Plant Growth

FARZANA YASMIN¹, RADZIAH OTHMAN², RASHID MA³, REDDY BN⁴, NAZMUL MHM⁴

¹Faculty of Science, Lincoln University, 12-18, Jalan SS6/12, Off JalanPerbandaran, 47301 Petaling Jaya, Selangor Malaysia

²Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

³Faculty of Medicine, UniversitiTeknologi MARA, Sungai Buloh, 47000 Selangor, Malaysia.

⁴Graduate School of Medicine, Perdana University, Jalan MAEPS Perdana, Serdang 43400, Selangor, Malaysia

Correspondence to Nazmul MHM, Graduate School of Medicine, Perdana University, Jalan MAEPS Perdana, Serdang 43400, Selangor, Malaysia. Email: poorpiku@yahoo.com

ABSTRACT

Aim: Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that improve plant growth by a wide variety of mechanisms. This study was conducted to observe the effect of Rhizobacterial isolates on early growth of sweet potato plants.

Methods: A pot experiment under glasshouse conditions was conducted to observe early growth of plant as affected by bacteria. The inoculation process could stimulate the plant growth and development as observed in the smaller pots (2kg soil). Plants were treated with PGPR isolates and harvested after 30 days of growth.

Results: Four bacterial isolates produced PGP compounds like IAA, solubilized phosphate, fixed nitrogen and resistance to antibiotic. Most of isolates resulted in a significant increase of root and shoot growth. A significant increase of root volume (58%) and root dry weight (56%) was recorded as compared to uninoculated control. The results showed that inoculation of *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 increased tops and roots growth, uptake of N, P, K Ca and Mg in plant tissue and bacterial population in soil.

Conclusion: The experiment indicated that *Klebsiella* sp and *Erwinia* sp used as bioenhancers might be beneficial for growth of sweet potato.

Key words: Sweet potato, Plant growth-promoting rhizobacteria, *Klebsiella* sp and *Erwinia* sp.

INTRODUCTION

Sweet potato is an important starchy root crop grown throughout the tropical and sub-tropical countries. It is a very nutritive vegetable, producing substantially high energy per hectare per day compared to rice, wheat, maize and cassava. Sweet potato requires high input of chemical fertilizers for commercial production. Chemical fertilizers can reduce the soil microbial flora and fauna which also cause environmental pollution, water pollution, health problems as well as increase the cost of production (Umair et al., 2018). Plant growth-promoting Rhizobacteria (PGPR) are a group of bacteria that colonize the rhizosphere and provide direct or indirect effect of the crop plants. It has been found that PGPR significantly enhanced plant and root growth and plant nutrition as well as yield of many crops (Compant and Sessitsch, 2010 and Ashok et al., 2015). PGPR shows promising results by making nutrients more available (e.g. by solubilization of phosphates) or increasing plant access to nutrients (e.g. by increasing root surface area). PGPR strains were observed to enhance the uptake of nutrient and yield in corn, wheat, sorghum and rice plants which are the major contributors for beneficial effects of PGPR to plants. (Ashrafuzzaman et al., 2009 and Das et al., 2013).

Dawwam et al., (2013) isolated different PGPR strains from sweet potato roots. Seven Rhizobacterial (PGPR) strains were tested for IAA producing and Phosphate Solubilizing activity. All isolates were able to produce IAA while four isolates solubilized rock phosphate. These isolates increased nutrient uptake as well as increased shoot and root dry weights. Plant growth improvement by PGPR could be due to mechanisms such as the ability to produce hormones like indole acetic acid, gibberellic acid and cytokinins, symbiotic nitrogen fixation, antagonism against phytopathogenic microorganisms and

solubilization of mineral phosphates and mineralization of other nutrients (Bashan et al., 2014). Therefore, an experiment was conducted to observe the effect of Rhizobacterial isolates on early growth of sweet potato plants.

MATERIALS AND METHODS

Vine cuttings of sweet potato was grown in two kg of sterilised sandy soil. The bacterial treatments consisted of: i) Control, ii) *Klebsiella* sp. UPMSP9 iii) *Erwinia* sp. UPMSP10 iv) *Azospirillum* sp. SP7 and v) *Bacillus* sp. UPMB10. An inoculum concentration of 10⁹cfu/ml was applied into the respective pots. Each pot was inoculated at planting and two weeks after planting with five ml inoculum/pot/application. The pots were covered with black polythene bag to prevent direct contamination. Hoagland's solution (Hoagland, 1950) was then added into each pot every two days to supply plants with the essential plant nutrients. All plants were watered daily with distilled water as required. The experiment was run in a Completely Randomized Design (CRD) with three replications. Plants were harvested at 30 days of growth.

At the harvest time sweet potato shoots (leaves and stems) were separated from the whole roots and soil and their fresh weights were determined. The shoots were placed into brown paper bags and oven dried at 70°C for two days and the dry weights were recorded. Fibrous roots and storage roots were separated from the soil, washed and cleaned from adhering soil and their fresh and dry weights were determined. Fresh soil was sampled for total bacterial population and the rest was air dried for nutrient content analysis. Root volume was determined by using the water displacement method.

Dried shoots were ground using electric grinder and passed through 0.5 mm sieve. Shoot samples were digested with concentrated sulphuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) following the micro-kjeldahl method (Thomas *et al.*, 1967). N, P, K concentrations were determined by using autoanalyser and Ca, Mg by using Atomic Absorption Spectrophotometer. The total population of bacteria in soil was determined at harvest by using total plate count technique (Parkinson *et al.*, 1971). The concentrations of IAA in soil were measured using modified method of Sarwar *et al.*, (1992). Soil pH was measured with a glass electrode pH meter (PHM 210, Metrolab) in a 1:2.5 soil-water suspension. The total nitrogen was determined following the micro-Kjeldahl method (Bremner, 1982). The available phosphorus was measured by Bray-2 method (Bray and Kurtz, 1945). Concentrations of exchangeable K, Ca and Mg were determined using the shaking method (Schollenberger and Simon, 1945).

Statistical Analysis: Analysis of Variance (ANOVA) were run using Statistical Analysis System (SAS, version 6.12, 1989). and treatments means were compared using Tukey Studentized Range (HSD) test at $p=0.05$.

RESULTS AND DISCUSSION

Plant growth: Significant difference in the plant growth were observed between the bacterial strains tested (Table 1). Plants treated with *Klebsiella* sp. UPMS9 and *Erwinia* sp. UPMS10 showed significantly higher shoot and root dry weights and root volume compared to uninoculated control. These could be due to the ability of the bacteria to produce IAA (auxin) and other plant growth regulators activities. The IAA would promote root initiation, cell division and cell enlargement, which stimulated growth and development of roots and morphological and physiological changes in inoculated plant roots (Rathaur *et al.*, 2012 and Ashraf *et al.*, 2011). Previous studies showed that the use of indole acetic acid (IAA) producing PGPR significantly increase in plant height, root length and dry matter production of shoot and root of rice seedlings (Ashrafuzzaman *et al.*, 2009). Rani *et al.*, (2012) isolated sixty Rhizobacteria which produced indole acetic acid (IAA) and other PGPR traits. PGPR isolates influenced seed germination, shoot length, root length, dry matter production of shoot, nodule number and nodule mass of pigeon pea.

Nutrient Uptake in Shoot: Inoculation with rhizobacteria significantly ($p<0.05$) increased the nutrient uptake in sweetpotato shoots (Table2). Plants inoculated with *Klebsiella* sp. UPMS9 and *Erwinia* sp. UPMS10 seemed

to show higher uptake of N, P, K, Ca and Mg compared to the uninoculated control. Improved plant growth with inoculation was due to increased uptake of N, P, and K of the plants. The enhanced uptake of essential nutrient could be through stimulation of root growth and development of the plants which subsequently increased dry matter and accumulation of minerals in stems and leaves (Gravelet *et al.*, 2007 and Kumar *et al.*, 2014). The beneficial effect of bacterial inoculation on sweet potato has been observed by Radziah and Tan (1999) and Farzana *et al.*, (2009). They observed more plant growth and nutrient uptake in inoculated sweet potato plants compared to uninoculated plants. Biostimulant species of *Pseudomonas* and *Bacillus* can produce phytohormones or growth regulators that cause crops to have greater amounts of fine roots which have the effect of increasing the absorptive surface of plant roots for uptake of water and nutrients (Sharma *et al.*, 2012).

Population of Bacteria and Nutrient Concentrations in Soil: The PGPR inoculation significantly ($p<0.05$) influenced bacterial population, N, P, Ca, Mg and IAA concentration in the soil (Table 3 and Table 4). Soil inoculated with *Klebsiella* sp. UPMS9 showed the highest total bacterial populations, soil pH, Mg and IAA concentrations. Higher nutrients concentrations may also be due to the supplied nutrient solution (Hoagland solution). Other studies have shown that single or dual inoculation of wheat seedlings with PGPR in sterilized soil resulted in significantly increased nutrient contents in soil (Dinesh R and Ghoshal Chaudhuri S, 2013). Higher IAA concentrations in the soil probably due to the bacteria synthesized IAA through TRP pathways (Dinesh *et al.*, 2010, Kumar *et al.*, 2015). However, there was presence of bacteria in the uninoculated control soil even though the soil used was sterilized. This could be due to the contamination from the dust in the glasshouse. Higher population of total bacteria was observed in soil inoculated with *Klebsiella* sp. UPMS9 and *Erwinia* sp. UPMS10. These bacteria could positively interact with plants roots and enhance plant growth. Compounds present in root system of sweet potato plant would affect the composition and activity of microbial population in the rhizosphere. Plants growth influence microbial population by the secreting plant exudates, which consist of several organic compounds such as sugars, amino acids, vitamins, tannins, alkaloids, phosphatides and other unidentified substances (Dinesh *et al.*, 2012 and Xing *et al.*, 2014). The simple sugars could provide readily available sources of carbon and energy for microbial growth that encouraged proliferation of other indigenous rhizobacteria.

Table 1: Effect of Rhizobacterial Inoculation on Growth Parameters of sweet potato plants

Treatments	Shoot Dry Weight(g Plant ⁻¹)	Root Dry Weight(g Plant ⁻¹)	Root Volume(cm ³)	Shoot to Root Ratio(S/R)
<i>Klebsiella</i> sp.	7.54 ^a	2.48 ^a	17.74 ^a	3.04 ^{ab}
<i>Erwinia</i> sp.	6.94 ^{ab}	2.38 ^a	16.74 ^a	3.14 ^{ab}
<i>Bacillus</i> sp.	5.69 ^c	2.16 ^a	16.15 ^a	2.61 ^c
<i>Azospirillum</i>	6.16 ^{bc}	2.16 ^a	13.73 ^b	2.90 ^{bc}
Control	5.50 ^c	1.59 ^b	11.21 ^c	3.45 ^a

Note: Means followed with same letter (s) in column are not significantly different ($P>0.05$)

Table 2: Effect of Rhizobacterial Inoculation on Uptake of N, P, K, Ca, and Mg in Shoots

Treatments	Nutrients Uptake (mg plant ⁻¹)				
	N	P	K	Ca	Mg
<i>Klebsiella</i> sp.	203.45 ^a	26.63 ^a	322.93 ^a	84.99 ^{ab}	33.52 ^a
<i>Erwinia</i> sp.	184.39 ^{ab}	24.87 ^a	272.13 ^b	87.74 ^a	30.71 ^a
<i>Bacillus</i> sp.	138.02 ^{cd}	19.70 ^b	193.08 ^{cd}	59.44 ^c	25.45 ^{bc}
<i>Azospirillum</i>	165.51 ^{bc}	20.19 ^b	236.19 ^{bc}	73.01 ^b	29.18 ^{ab}
Control	122.93 ^d	13.55 ^c	169.13 ^d	55.68 ^c	20.99 ^c

Note: Means followed with same letter (s) in column are not significantly different (P>0.05)

Table 3: Effect of Rhizobacterial Inoculation on Population of Bacteria and Concentration of IAA in Soils

Treatments	Population of Bacteria (log ₁₀ cfu g ⁻¹ soil)	Concentration IAA in Soil (mg kg ⁻¹)
<i>Klebsiella</i> sp.	5.02 ^a	0.246 ^a
<i>Erwinia</i> sp.	4.97 ^{ab}	0.070 ^b
<i>Bacillus</i> sp.	4.91 ^c	0.046 ^b
<i>Azospirillum</i>	4.96 ^{bc}	0.053 ^b
Control	4.72 ^d	0.036 ^b

Note: Means followed with same letter (s) in column are not significantly different (P>0.05)

Table 4: Effect of Rhizobacterial Inoculation on soil pH and Nutrient Concentration

Treatments	Nutrients Concentration					
	pH	(%) N	(+) mg kg ⁻¹ P	(+) mg kg ⁻¹ K	(+) mg kg ⁻¹ Ca	(+) mg kg ⁻¹ Mg
<i>Klebsiella</i> sp.	7.60 ^a	0.01 ^a	23.89 ^{ab}	0.03 ^a	0.52 ^{bc}	0.08 ^a
<i>Erwinia</i> sp.	7.52 ^{ab}	0.01 ^a	26.09 ^a	0.03 ^a	0.57 ^{ab}	0.04 ^c
<i>Bacillus</i> sp.	7.57 ^{ab}	0.01 ^a	24.36 ^{ab}	0.03 ^a	0.57 ^{ab}	0.06 ^b
<i>Azospirillum</i>	7.59 ^{ab}	0.01 ^a	22.63 ^{ab}	0.03 ^a	0.65 ^a	0.06 ^b
Control	7.43 ^b	0.004 ^b	21.29 ^b	0.02 ^a	0.45 ^c	0.04 ^c

Note: Means followed with same letter (s) in column are not significantly different (P>0.05)

CONCLUSION

In conclusion, our result suggested that PGPR inoculation significantly increased the early growth of the sweetpotato plants under pot experiment. PGPR are highly beneficial for plant growth and can serve as potential bioenhancers. Plants inoculated with *Klebsiella* sp. UPMS9 and *Erwinia* sp. UPMS10 increased growth (tops and roots) and nutrient uptake (N, P, K, Ca and Mg) of the sweetpotato plants. Improved plant growth was related to the improved soil chemical and microbial properties.

REFERENCES

- Ashok Kumar, Bahadur, B. R. Maurya, R. Raghuwanshi, V.S. Meena, D. K. Singh and J. Dixit .2015. Does a Plant Growth Promoting Rhizobacteria Enhance Agricultural Sustainability? Journal of Pure and Applied Microbiology 9(1):715-724.
- Ashraf Muhammad Arslan, Rasoo Mahmood and Mirza Muhammad Sajjad. 2011. Nitrogen Fixation and Indole Acetic Acid Production Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharum officinarum* L.). Advances in Biological Research 5(6): 348-355.
- Ashrafuzzaman M, HossenFarid Akhtar, Ismail M Razi, Hoque Md. Anamul, Islam M. Zahurul, Shahidullah and Meon Sariah. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. African Journal of Biotechnology 8(7): 1247-1252.
- Bashan, Y., Bashan, L. E., Prabhu, S. R. and Hernandez, J. B. 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives. Plant and Soil 378: 1–33.
- Bray, R.H., and Kurtz, L. T. 1945. Determination of total organic, and available forms of phosphorus in Soils. Soil Science 59: 39-45.
- Bremner, J.M. and Mulvaney, C. S., 1982. Nitrogen-total. In: *Method of Soil Analysis*, ed. Pace, A. L., Agronomic Monograph No.9, American Society of Agronomy, Madison, Wisconsin, USA, pp 595-624.
- Compant S, Clément S, Sessitsch A. 2010. 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(5): 669-678.
- Das, A.J., Kumar, M., Kumar, R., 2013. Plant growth promoting rhizobacteria (PGPR): An alternative of chemical fertilizer for sustainable, environment friendly agriculture. Research Journal of Agriculture and Forestry Sciences 1(4) :21-23.
- Dawwam G.E., Elbeltagy, A., Emar, H.M., Abbas, I.H., Hassan ,M.M. 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato. Plant. Annals of Agricultural Science 58(2),195-201.
- Dinesh R, Ghoshal Chaudhuri S. 2013 Soil biochemical/ microbial indices as ecological indicators of land use change in mangrove forests. Ecological indicators 32:253–258.
- Dinesh R, Srinivasan V, Hamza S, Manjusha A .2010, Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Curcuma longa* L.)]. Bioresource technology, 101:4697–4702.
- Dinesh R, Srinivasan V, Hamza S, Manjusha A, Sanjay Kumar P. 2012. Short-term effects of nutrient management regimes on biochemical and microbial properties in soils under rainfed ginger (*Zingiber officinale* Rosc.). Geoderma 173–174:192–198.
- Farzana Y, Saad S, Radziah O and Kamaruzaman S. 2009. Growth and storage root development of Sweet potato inoculated with rhizobacteria under glasshouse conditions. Australian Journal of Basic and Applied Sciences 3(2): 1461-1466.
- Gravel V, Antoun H, Tweddell RJ. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol/Biochem 39:1968–1977.

15. Hoagland, D. R. and Arnon, D. I. 1950. The water culture method for growing plants without soil. California. Agriculture Experiment Station Service Manual 35. 143 pp.
16. Kumar, A., Maurya, B. R., Raghuwansh, R. . 2014. Isolation and Characterization of PGPR and their effect on growth, yield and Nutrient content in wheat (*Triticumaestivum* L.). Biocatal. Agril. Biotechnol.3: 121-128.
17. Parkinson, D. T., Gray, R. G. and Williams, S. T. 1971. Methods for studying the Ecology of soil Microorganisms IBP handbook no. 19.
18. Radziah, O. and Tan. L. O. 1999. Effect of *Azospirillum* and L-tryptophan on soil IAA and growth of sweetpotato. In Proceeding Soil Science Conference of Malaysia 1999. (Editors. Zauyah, S., A. B. Rosenani. M.S. Halimi) MSSS. UPM. 219-230.
19. Rani M. Usha, Arundhati and Reddy Gopal. 2012. Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. African Journal of Biotechnology 11(32): 8085-8091.
20. Rathaur Preeti, Raja Waseem, Ramteke P W and John S A. 2012. Effect of UV-B Tolerant Plant Growth Promoting Rhizobacteria (PGPR) on Seed Germination and Growth of *Withaniasomnifera*. Advances in Applied Science Research 3(3):1399-1404.
21. Sarwar, M., Arshad, M., Martens, D. A. and Frankenberger, Jr. W. T. 1992. Tryptophan-dependent biosynthesis of auxins in soil. Plant and Soil 147: 207-215.
22. SAS Version 6.12. 1989. SAS/ STAT. Guide to Personal Computers. SAS Institute Inc., Cary, North Carolina.
23. Schollenberger, C. J. and Simon, R. J. 1945. Determination of exchange capacity and exchangeable base in soil ammonium acetate method. Soil Science 59: 13-23.
24. Sharma B C, Subba R and Saha A. 2012. In vitro solubilization of tricalcium phosphate and reduction of IAA by phosphate solubilizing bacteria isolated from tea rhizosphere of Darjeeling Himalaya. Plant Sciences Feed 2(6): 96-99.
25. Thomas, R. L., Sheard, R. W. and Moyer, J. R. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. Agronomy Journal 59:240-243.
26. Umair Mehmood, Muhammad Inam-ul-Haq, Muhammad Saeed, Adeela Altaf, Farooq Azam. 2018. A brief review on plant growth promoting rhizobacteria (pgpr): a key role in plant growth promotion. Plant Protection, 2 (2): 77-82.
27. Xing F. H, Jacqueline M. C, Kenneth F. R, Ruifu Z, Qirong S, Jorge M. V. .2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92(4): 267-275.