ISSN: 2278-778X
Research Article
www.ijbio.com

PGPR *BACILLUS* SPECIES ISOLATED FROM TOMATO PLANT–A COMPARATIVE STUDY ON COCONUT WATER ENRICHMENT

OS Aysha, P Vinothkumar*, S Vasuki, S Valli, P Nirmala, A Reena
PG & Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600 119 (T.N.) India

***Corresponding Author:** Dr. P. Vinothkumar, PG & Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600 119 (T.N.) India

Received for publication: June 15, 2012; **Accepted**: September 29, 2012.

Abstract: Plant growth promoting rhizobacteria (PGPR) are bacteria that colonize plant roots, they promote plant growth and reduce disease or insect damage. PGPR have been identified within many different bacterial taxa, most commercially developed PGPR for agricultural crops are species of *Bacillus* which form endospores that confer population stability during formulation and storage of products. Here the rhizobacteria *Bacillus* sp has been isolated from tomato plant and characterized with routine biochemical tests. Then the isolated rhizobacteria was enriched by inoculating with coconut water as carrier medium. The growth pattern was studied with the bacterial strain that was aseptically inoculated within coconut water in flask, coconut water within intact coconut and in nutrient broth. The rhizobacterial culture inoculated within coconut water in coconut has multiplied to the tune of 10.0×10^{-6}cfu ml^{-1}within a period of 24 hrs. The bacterial strain developed mucoidal colonies on coconut water agar medium as a result of increased polysaccharide production. Seed colonization and plant growth promotion of tomato plant was more when culture was grown in coconut water was used for seed treatment.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), *Bacillus* sp, Coconut Water, Colonization.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are naturally occurring bacteria soil bacteria that aggressively colonize plant roots and benefits plants by providing growth promotion. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass. Production through direct effects on root and shoots growth.[20]Various bacteria which are predominantly studied and increasingly marketed as the biological control agents includes genera Such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agrobacteria* exposure to PGPR triggers a defense response by the crop as if attacked by pathogenic organisms. PGPR benefits plants by providing growth promotion.[18]Among the bacilli, strains of *B. subtilis* are the most widely used PGPR due to their disease reducing and antibiotic producing capabilities when they applied as seed treatments.[6]*Bacillus* sp. is one of the most potential genera due to their spore forming ability, thereby increasing the adaptation of *Bacillus* strain to commercial formulation and field application. *Bacillus subtilis* is known to positive influence on plant growth, vitality and the ability of the plant to cope with pathogens often resulting in higher yield. *B. mucilaginous* has been observed for its capability in solubulizing potassium[7].

There are several PGPR inoculants currently commercialized that seems to promote plant growth through at least one mechanism: suppression of plant disease (termed Bioprospectants), improved nutrient acquisition (termed Biofertilizers) or phytohormone production (termed Biostimulants)[22]. Inoculants development has been most successful to deliver biological control agents of plant diseases i.e. organisms capable of killing other organisms pathogenic or disease causing to crops. Various bacteria which are predominantly studied and increasingly marketed as the biological control agents includes the genera such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agro bacterium*. They suppress the plant disease through at least one mechanism; induction of systemic resistance, and production of siderophores or antibiotics. *B. subtilis* as well as other *Bacilli* are potentially useful as bio-control agents [10]

The carrier is the delivery vehicle of live microorganisms from the factory to the field; however no universal carrier or formulation is presently available from the release of microorganisms in to the soil.[24]A cheap and locally available materials for cultivation and as carrier for formulation has resulted in use of several naturally occurring plant derived from organic substrates. Such as coconut water (CW) present in the coconut was free from any microbial contaminants and

is highly nutritious, rich in amino acids, vitamins and minerals[16]. Coconut water is known to induce plant growth in tissue culture and is frequently used as a growth supplement in plant tissue culture media due to its rich content of nutrients and plant growth regulators. One way to enhance the effect of CW is to competitive with PGPR. Studies have been carried out indicating that PGPR can be used to increase crop yields [15]. It has been shown that CW also acts as a good nutritional supplement in many of the bacteriological media[25]. Microbial formulations are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application. They are designed to improve soil fertility and help plant growth by increasing their numbers their biological activity in the root environment. PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides, and supplements[21]. Plant growth-promoting rhizo-bacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Here, we have isolated and characterized the PGPR from the rhizosphere soil of rice field for the enhancement of growth of rice.[17]

A simple method for multiplication of PGPR in coconut water and within coconut is described here. This method is expected to help farmers easily multiply PGPR, for usage in nurseries of transplanted plants and agricultural crops.

MATERIALS METHODS

Isolation of PGPR Bacillus strain from soil sample:

The soil samples were collected from the rhizosphere of healthy tomato growing agriculture fields around Trichy district. Both the rhizosphere soil and root samples were taken from the depth of 0- 15 cm. The soil samples were brought in polythene bags and stored in cool place to maintain their physiochemical properties for further use. The soil samples were serially diluted up to 10^{-9} dilutions. The dilutions were plated on nutrient agar plates. The plates were then incubated at 37°C for 24 hrs. The suspected colonies were picked up, sub-cultured and preserved on Nutrient agar slants at 4-5°C.

The isolates were identified by routine biochemical tests as per the guidelines of Berge's Manual of Determinative Bacteriology [9]

Cultivation of PGPR strains Bacillus sp. on coconut water agar medium:

Colony morphology of bacterial culture on Coconut water agar medium: Plain agar (20ml) with double the agar concentration (agar at 30g/l) was added to 20ml of double distilled water, and sterilized by autoclaving. Coconut water (CW) was collected aseptically with sterile 20ml syringe fitted with 18 gauge needle, by inserting it through the opened eye of the coconut aseptically. 20ml of coconut water was further filter and sterilized with 0.2µ disposable syringe filter and collected in 100ml conical flask. Sterile molten plain agar was added to above coconut water in 100ml conical flask and poured into sterile plates. *Bacillus* culture was streaked on the plates and incubated at 28°C. Colony morphology was observed and compared with nutrient agar plates and coconut water agar plates.

Enrichment of PGPR Bacillus sp within coconut water in flask, within coconut and nutrient broth:

PGPR *Bacillus* sp. was enriched within coconut water[2]and inoculation of *Bacillus* sp (PGPR) into coconut water in conical flask. Eye of the coconut was surface sterilized with 70% alcohol and removed with help of sterile blade. Coconut water was aseptically collected and transferred to a sterile conical flask with help of sterile 20ml syringe fitted with 18 gauge needle by inserting it through opened eye of coconut. 20ml of filtered sterilized with 0.2µl disposable syringe filter and collected in an100ml sterile conical flask. A single colony of bacterial culture was suspended in 1ml sterile distilled water from that 100µl of suspension was transferred to coconut water. It was incubated at 28°C for 24-48 hrs without shaking. Samples were drawn from coconut water suspension at regular time intervals and cell count was monitored using viable plate count method on nutrient agar plates. The same method was followed and the *Bacillus* species was inoculated in nutrient broth. Then cell count was monitored at regular time intervals which served as control.

Tomato Seed treatment and ability of colonization comparing with Bacillus culture grown in coconut water and nutrient broth:

Seed treatment was done to compare the colonizing ability of *Bacillus* culture on tomato seeds grown in coconut water and nutrient broth. Seeds of tomato were surface sterilized with 1% sodium hypochlorite for 30 min and washed in sterile distilled water aseptically and spread on sterile tissue paper to drain excess water. Surface sterilized seeds were then soaked in *Bacillus* culture in coconut water and nutrient broth for 30 min. Treated seeds were kept in sterile plates at room temperature. Bacterial population on seeds was determined at 30mts and 24hrs after seed treatment, by serial dilution with coconut water and nutrient broth culture. Dilution plating was done on nutrient agar plate, and total viable count was measured as CFU/seed.

Pot Culture Method:

The PGPR *Bacillus* culture grown in different media was assessed under pot culture method. The isolated bacterial culture was grown in nutrient broth coconut water; intact coconut and seed treatment were also done. Soaking seeds into sterile distilled water without inoculation of bacterial culture was served as control. Seeds were sown in sterile soil mixture (1:1) in big plastic cups and the planting medium was sterilized by autoclaving at 121°C for 20min for 3days. Then the plants were watered regularly with sterile water and no chemical fertilizers are applied externally. After 28 days of planting, the seedlings were uprooted for observation.

Statistical analysis of data

Data was analyzed by one way analysis of variance (ANOVA) followed by fischer's LSD post hoc test using spss 10.0 software (spss Inc., Chicago). The values are expressed as mean ±.

RESULTS AND DISCUSSION

Rhizosphere is a rich habitat of microorganisms and should be explored for obtaining potential PGPR, which can be useful in developing bio inoculants for the enhancement of growth and yield of crop plants. The beneficial effect of plant growth promoting rhizobacteria, particularly those belonging to the genus *Bacillus* or *Pseudomonas*, in enhancing growth and overall plant establishment is well established.[10]Plant growth promoting bacteria are important in managing plant growth because of their effects on soil conditions, nutrient availability and tree growth.[11]Therefore, it is necessary to develop efficient strains in field conditions. Isolation and identification of rhizosphere microorganisms such as Pseudomonas and Bacillus for proper utilization of their beneficial effects as plant growth promoters on maize, wheat, rye and other crops have been well reposrted.[14]Since PGPR application on agriculture is currently attracting many attentions of the community. The present study was undertaken to isolate and analyze the PGPR enrichment with coconut water.

The colony morphology of the isolate was large, flat and opaque colonies were observed. The bacterial isolates on nutrient agar media were further subjected to biochemical and physiological characterization and the results obtained are presented in Table-I.

Growth characters of bacterial culture on coconut water agar was small round, mucoidal appearance than those on nutrient agar were large, flat, undulated colonies with dry appearance.

The PGPR strain *Bacillus* sp. was found to multiply well in coconut water utilizing it as sole source of nutrients. The PGPR strain was grown in coconut water collected in conical flask as well as within intact coconut. In nutrient broth which served as control had maximum bacterial count 8.8×10^{6} CFU/ml at dilution factor 10^{-6} within 24hrs incubation and it declines over at 48hrs, whereas in coconut water in flask, the maximum population was11.0×10^{6} CFU/ml attained within a period of 24 hr and then the growth was declined at 48 hrs. The population of the bacterial suspension in coconut water within coconut was 10.0×10^{6} CFU/ml at the same dilution factor of $10^{-6}$and it declines at 48hrs with further incubation. Hence maximum population of PGPR isolate was observed in coconut water medium than nutrient broth at 24 hr incubation and the growth of the organism declined at 48 hrs incubation due to difference in pH of coconut water (Table II, III, IV).

Assessment of the bacterial population on treated tomato seeds showed that cells from the culture grown in coconut water were present in more in numbers on the seed as compared to those treated with culture grown in nutrient broth. The initial population of the culture on tomato seed in the nutrient broth was 6.6×10^{6} CFU/ml at 10^{-6} dilution factor, after 30 min of tomato seed inoculated with the culture was subjected to viable count method showed that 8.5×10^{6} colony forming unit/ml at 10^{-6} dilution plate. After 24hr incubation the bacterial count on the seed was counted as 7.2×10^{6} CFU/ml at 10^{-6} dilution factor, whereas the initial population count of the culture in coconut water with tomato seed had 7.8×10^{6} CFU/ml at 10^{-6} dilution plate. After 30min of incubation the higher bacterial count on treated tomato seed was 10.0CFU/ml $\times10^{6}$ at same dilution factor and at 24 hr incubation the population of cell culture was increased to 11.4 CFU/ml $\times10^{6}$ of the cell suspension in 10^{-6} dilution factor were illustrated in, Table V.

Comparison of the seed treatment revealed that seeds treated with PGPR grown in coconut water had more ability to promote plant growth than those treated with bacteria grown in nutrient broth. The treatment of tomato seeds with PGPR *Bacillus* culture in coconut water compare within coconut had totally 16 numbers of leaves, the height of the plant was measured upon 3.5cm with 0.35g as fresh shoot and 0.85g as root weight of the tomato plant. Those seeds treated with *Bacillus* culture in nutrient broth had totally 12 numbers of leaves with 2.3cm as plant height and the fresh shoot and root weight was measured as 0.21g and 0.53g. The untreated tomato seeds had the least count with totally 9 numbers of leaves, the height of the plant measured as 1.6cm and the fresh shoot and root weight was weighed and recorded as 0.06g and 0.14g in Table VI.

In the present investigation PGPR *Bacillus* sp. were isolated from rhizosphere soil sample of tomato plant.

Similar results on the occurrence of *Bacillus* spp. in the rhizosphere soil has been reported by various workers[4,5]. The isolated culture were further processed and identified based on morphological and biochemical characters by referring to Berge's Manual of Determinative Bacteriology (Table-I). The growth characters of bacterial culture on coconut water agar medium was small round, mucoidal appearance than those compared with the colony morphology on nutrient agar medium were large undulated with dry appearance. Similar observation was done by (Anith, 2009) with the colonies of *Bacillus pumilis* strain SE34 on coconut water agar were flat, spreading with mucoidal appearance and those on nutrient agar were small, well separated and having dry appearance**.** The present study is in accordance with the earlier findings of Bianciotto, 2008 and Jofre, 2004 had proved that the seed and root colonization efficiency was highly influenced by the type and amount of bacterial polysaccharides.

Table I: Biochemical identification of PGPR isolate *Bacillus* sp.

Characteristics	Results
Gram's staining	Gram positive
Spore staining	Positive
Motility	Motile
Catalase test	Positive
Oxidase test	Negative
Starch utilization test	Positive
Indole	Negative
Methyl red	Negative
Vogesproskauer	Positive
Citrate	Positive
Nitrate	Positive
Glucose	Acid production
Lactose	Acid production
Mannitol	Acid production
Dextrose	Acid production
Sucrose	Acid production
Xylose	Acid production

The multiplication of PGPR strain *Bacillus* sp within coconut water as carrier attained a maximum population of 10.0×10^6cfu/ml at 24 hrs incubation on further incubation the growth rate of the culture declines. In case of multiplication of bacterial culture in coconut water in flask had 11.0×10^6 cfu/ml at 24hr incubation (Table 2, 3 & 4). This result is correlated with the work of Anith, 2009, on the growth pattern of bacterial strain *B. pumilus* SE34, multiply well in coconut water and maximum population was attained within a period of 24 hr and then it declined. The population of the bacterial strain was 10^8 cfu/ml in case of coconut water within coconut and 10^9cfu/ml in the case coconut water in flask [19]studied that coconut water was rich in source of nutrients for the multiplication of microbial agents. It has been shown that coconut water also acts as a good nutritional supplement in many of the bacteriological media.

Table II: Multiplication and growth of *Bacillus* sp. In Nutrient broth (Total viable count method)

Dilution factor	(CFU/ml)* after 24 hrs	(CFU/ml)* after 48hrs
10^{-6}	8.8×10^6	7.1×10^6
10^{-7}	7.0×10^7	5.3×10^7
10^{-8}	5.6×10^8	4.0×10^8

***** Mean of three independent observations.

Table III: Multiplication and growth of *Bacillus* sp., in Coconut water conical flask

Dilution factor	(CFU/ml)* after 24 hrs	(CFU/ml)* after 48hrs
10^{-6}	11.0×10^6	9.8×10^6
10^{-7}	9.3×10^7	7.7×10^7
10^{-8}	7.5×10^8	6.0×10^8

*****Mean of three independent observations.

Table IV: Multiplication and growth of *Bacillus* sp. in Coconut water within coconut

Dilution factor	(CFU/ml)* after 24 hrs	(CFU/ml)* after 48hrs
10^{-6}	10.0×10^6	8.2×10^6
10^{-7}	7.8×10^7	6.4×10^7
10^{-8}	6.0×10^8	5.5×10^8

*****Mean of three independent observations.

In current study the bacterial population on tomato seeds treated with coconut water showed 11.4×10^6cfu/ seed after 24 hr incubation. The mucoidal nature enriched by content of coconut water helped in better agglutination of the cells to the seed surface (Table v). This correlates with the experimental results of Anith, (2009), reported that the tomato seeds treated with PGPR strain *Bacillus*SE34 grown in coconut water had resulted in maximum bacterial population of 8.7×10^6cfu/seed after 24 hr incubation. In earlier studies, The tomato seeds treated with *B. subtilis* culture containing 10^6cfu/ml to promote the tomato plant growth was experimented by [1]surface sterilized the wheat seeds by soaking seeds in liquid culture medium for 1 hr using 10% gum arabic as adhesive agent. The microbial population of *Bacillus* sp. was 2×10^8cfu per seed. The seed treatment was done with PGPR *Bacillus* sp. as inoculant carrier and coconut water as carrier medium, to enhance the plant growth promoting activity of PGPR *Bacillus* sp**.**

The plant growth parameters of tomato seeds treated with PGPR *Bacillus* sp. with coconut water as carrier medium resulted in maximum number of leaves (16 no. of leaves) with increased plant height (3.5cm) and fresh shoot and root weight (0.35g and 0.85g). When compared, the tomato plant growth of seeds treated with nutrient broth culture and untreated seeds had least number of leaves with decreased plant height and fresh shoot and root weight (Table VI). The results of the present study are in accordance with the results of the study by Woyessa[27] had reported that on inoculation of AURB65 (*Bacillus subtilis*), the amount of fresh shoot and root weight (0.25g) of the plant variety had been increased when compared with

Burkholderiacepacia[23]. Anith, (2009), reported that plant growth parameters of tomato seeds treated with *Bacillus* strain SE34 grown in coconut water resulted in maximum numbers of leaves as well as increased fresh shoot (0.22g) and root (0.025g) weight of the plant when compared with the treated seeds in nutrient broth. The current study results correlates with the results of recent experimental findings, Kim,[13] demonstrated that PGPR strains *Bacillus subtilis* had increased growth promotion of medicinal plant to 150% the plant height was measured as 50.4cm in length. The maximum plant height of chilli plant was recorded as 54.17 cm in the combined treatment of PGPR C2 and C32 isolate from rhizosphere soil of chill *(Capsicum annuum L.)* plant was shown by Wahyudi[26] studied on PGPR *Bacillus* sp. (Cr-69, Cr33 and Cr68) significantly promoted the length of primary roots and lateral roots respectively.

Table V: Bacterial population on tomato seeds (Total viable count method)

In Nutrient broth	Initial population in the suspension(CFU/ml)*	CFU/seed* after 30 min	CFU/seed* after 24hrs
10^{-6}	6.6×10^{6}	8.5×10^{6}	7.2×10^{6}
10^{-7}	4.4×10^{7}	6.3×10^{7}	6.1×10^{7}
10^{-8}	2.8×10^{8}	4.6×10^{8}	5.3×10^{8}
In Coconut water	**Initial population in the suspension(CFU/ml)***	**CFU/seed* after 30 min**	**CFU/seed* after 24hrs**
10^{-6}	7.8×10^{6}	10.0×10^{6}	11.4×10^{6}
10^{-7}	6.6×10^{7}	8.5×10^{7}	9.8×10^{7}
10^{-8}	4.7×10^{8}	6.0×10^{8}	7.4×10^{8}

*Mean of three independent observations.

Table VI: Plant growth parameters of tomato seedlings under sterile soil conditions

Treatment	No. of leaves	Plant Height (cm)*	Fresh shoot weight (g)*	Fresh root weight (g)*
Control	9±2.51	1.6±0.12	0.06±0.02	0.14±0.015
Nutrient broth	12±3.0	2.3±0.25	0.21±0.025	0.53±0.035
Coconut water	16±1.5	3.5±0.15	0.35±0.01	0.85±0.03

*Mean values of triplicates ± SD

The present results suggest that coconut water present in uncontaminated form within the intact coconut serves as excellent carrier medium for multiplication of plant growth promoting rhizobacteria that enhances the plant growth ability of various agricultural crops. The plant growth parameters such as plant height, number of leaves and weight of the shoot and root increases when the plant seedlings are treated with the rhizobacterial cultures formulated with coconut water as carrier medium.

CONCLUSION

Therefore, the result revealed that the presence of sucrose in coconut water had favored an increase in production of polysaccharides by the bacterial strains. The mucoidal nature of the colonies had positive influence on seed and root colonization. Assessment of the bacterial population on the treated tomato seeds showed that cells from the cultures grown in coconut water were present in more numbers on the seeds as compared to those treated with cultures grown in conventional broth.

Finally, this work reported that seed treatment with coconut water alone improved plant growth which was comparable with that observed in other bacterial treatments. The high population PGPR strain *Bacillus* sp. on tomato seeds at the time of seed treatment had positive effect on the plant growth promoting ability in tomato plant under sterile soil condition. This current report suggests that uncontaminated coconut water naturally available within the coconut rich in source of nutrients can be used as an enrichment medium for the multiplication PGPR rhizobacteria for their enhancement of plant growth conditions. It is a cheap and farmer friendly method for multiplication of Plant growth promoting rhizobacteria in coconut water within coconut. The inoculant production of low cost rhizobacteria promote plant growth (PGPR) is an alternative to reduce the use of pesticides and chemicals. Furthermore, the use of such inoculants can increase agricultural production, making the product more competitive and differentiated as well as reduced the costs to the producer, the less need for inputs. PGPR mediated agriculture was now gaining a worldwide importance and acceptance for an increasing number of crops and managed ecosystems as the safe method of pest control, agricultural development and establishment of good eco- friendly to the environment.

ACKNOWLEDGEMENTS

The authors are thankful to our management of Mohamed sathak group of educational institutions Chennai, Tamilnadu. We thank our dean, academic director and principal of our Mohamed sathak arts and Science College for providing us the infrastructure in our college and also for his valuable suggestion and constant encouragements. We thank our laboratory faculty members of our college for the help and support throughout the course of study.

REFERENCES

1. Adesemoye AO, Torbert HA, KloepperJW, Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system, Can. J. Microbiol., 2008; 54: 876–886.

2. Anith KN, Mature coconut as a bio-fermentor for multiplication of plant growth promoting rhizobacteria. Current Science, 2009; 97(11):1647-1653.

3. Bianciotto V, Andreotti S, BalestriniR, Bonfante P, PerottoS, Mucoid Mutants of the biocontrol strain Pseudomonas fluorescensCHA0 show increased ability in biofilm formation on mycorhizal and nonmycorrhizal carrot roots, Mol. Plant Microbe Interact, 2001; 14(2): 225-260.

4. Chakraborty U, Chakraborty B, Basnet M, Plant growth promotion and induction of resistance in Camellia sinensis by Bacillus Megaterium, J. Basic Microbiology, 2006; 46 (suppl 3): 186 –195.

5. Ferreira JTP, Santos TMC, AlbuquerqueLS, Santos JV, FilhoJAC, Neto CER. Isolation and selection of growth-promoting bacteria ofthe genus Bacillus and its effect on two varieties of lettuce (Lactuca sativa L.), Int. Res. J. Microbiol., 2011; 2(2):70-78.

6. Gutierrez Manero FJ, Probanza A, Ramos B, Colon Flores JJ, Lucas Garcia JA. Ecology, Diversity and Screening strategies of plant growth promoting Rhizobacteria (PGPR), J. Plantnutrition, 2003; 26 (sup.5):1101-1115.

7. Han HS, Supanjani, Lee KD. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant soil and Envt., 2006; 52(sup.3) :130-136.

8. Jofre F, Lagares A, Mori G. Disruption of dTDP-rhamnose biosynthesis modifies lipopolysaccharide core, exo-polysaccharide production, and root colonization in Azospirillumbrasilense, FEMS Microbiol. Lett., 2004; 231: 267-275.

9. John G, Host, Noel R, Krief, Peter H, Sneath A, Staley T, Stanley Williams T. Bergey's Manual of Determinative Bacteriology 1994; 9th edition. William and Wilkin's Baltimore U.S.A.

10. Joseph B, Patra RR, Lawrence R, Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicerorietinum L), Int J Plant production, 2007; 1(Suppl 2):141-152.

11. KarakurtHalil and AslantasRafet, Effects of some plant Growth promoting Rhizobacteria (PGPR) Strains on Plant Growth and Leaf nutrient content of apple, J. fruit and Ornamental plant research, 2010; 18(1): 101-110.

12. Khan MS, ZaidiA, Synergistic effects of the inoculation with Plant Growth- Promoting Rhizobacteria and an arbuscular mycorrhizal fungus on the performance of wheat. Turk. J. Agric., 2007; 31: 355-362.

13. Kim W, ChoWK, Chu H, Ryu KY, YunJC ParkCS. Genetic diversity of cultivable plant growth-promoting rhizobacteria in Korea. J. Microbiol. Biotechnol., 2001; 21(8): 777-790.

14. Luey M, Reed E, Glick BR. Applications of free-living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek., 2004; 86:1-25.

15. Matiru VN, Dakora FD, Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Africa Journal of Biotech., 2004; 3(1): 1-7.

16. Nandakumar R, Babu, Viswanathan R, Sheela J, Ragachander T, Samiyappan R, A new bio–formulation, containing plant growth promoting rhizobacterial mixture for the management of sheath bliqlet and enhanced grain yield in rice. Biocontrol, 2001; 46: 493-510.

17. Ashrafuzzaman M, FaridAkhtarHossen, Razi Ismail M, Md. AnamulHoque, Zahurul Islam M, Shahidullah SM, SariahMeon. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. Afr J Biotech., 2009; 8 (7): 1247-1252.

18. Pieterse CMJ, Pelt JA, Verhagen VWM, jurriaan T, Wees SCM, Leon Kloosterziel KM, Loon LC. Induced systemic resistance by Plant growth promoting rhizobacteria. Symbiosis, 2004; 35 (suppl 1-3): 39-54.

19. Prabakaran G, Hoti SL, Manonmani AM, Balram K, Coconut water as a cheap source for the production of endotoxin of Bacillus thuringenesis var. israelensis, a mosquito control agent. Acta. Tropica., 2008; 105:35-38.

20. Saharan BS, Nehra. Plant Growth Promoting Rhizobacteria: A critical review. Liv sciences and Medicine Res., Vol.2011; 1-30.

21. Shahidullah SM, Hanefi MM, AsharaFuzzaman M, Razilsmail, MA, Salam.Tillering dynamics in aromatic rice genotypes. Int J Agricultural and Biol., 2009; 11: 509-510.

22. Sunil E. Plant growth promoting rhizobacteria Microorganisms for agricultural development. 2008. Biological online on Nov 21.

23. Survase SA, Saudagar PS and. Singhal RS. Use of complex media for the production of scleroglucan by Sclerotiumrolfsii, MTCC 2156. Bioresour Technol, 2007; 98:1509-1512.

24. Trevors JT, Elsas, Van JD, Lee H, Overbeek, Van LS. Use of alginate and other carriers for encapsulation of microbial cell for use in soil. Microbial release, 1992; 1:61-69.

25. Unagal PC, Assantachai C, Phadungruengluij M, Suphantharika S, TanticharoenM, Verduyn C. Coconut water as a medium additive for the production of docosahexaenoic acid by Schizochtriummangroveisk 02. Bioresour. Technol., 2007; 98: 281-287.

26. Wahyudi AT, Astuti RP, Widyawati A, Meryandini A, Nawangsih AA, Characterization of Bacillus sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. J Microbiol and Antimicrobials, 2011; 3(2): 34-40.

27. Woyessa D, AssefaF, Effects of Plant growth-promoting rhizobacteria on growth and yield of tef (EragrostistefZucc. Trotter) under greenhouse condition. Res J of Microbiol., 2011; 6(4): 343-355.

Source of support: Nil
Conflict of interest: None Declared