Journal of Agricultural Technology 2012 Vol. 8(6): 2101-2113
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Plant growth promoting activity of nickel tolerant *Bacillus cereus* TS1

T. Sivakumar[1*], T. Shankar[2], P. Vijayabaskar[2] and V. Ramasubramanian[3]

[1]PG Research Department of Microbiology, Sivakasi-608124, Tamilnadu, India, [2]Department of Plant Biology and Plant Biotechnology, Sivakasi-608124, Tamilnadu, India, [3]Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-608124, Tamilnadu, India

T. Sivakumar, T. Shankar, P. Vijayabaskar and V. Ramasubramanian (2012) Plant growth promoting activity of nickel tolerant *Bacillus cereus* TS1. Journal of Agricultural Technology 8(6):2101-2113.

Keratinase producing bacterial strain was isolated from the chicken feather dumping site. The potential strain was identified by physical, biochemical characteristics, fatty acid methyl ester (FAME) and 16S rDNA sequences method. Rice seed treated with feather hydrolysates of *Bacillus cereus* TSI showed 100% of vigour index in plant growth activity due to secretion of IAA, siderophore, HCN and phosphatase were synthesized. In addition to that the *Bacillus cereus* TSI act effectively in fertilization process of soil. Even at a concentration of 300 mg nickel/gm of soil, the metal effect was detoxified by *Bacillus cereus*TS1 causing enhancement in shoot length, root length, chlorophyll content, fresh weight and dry weight.

Key words: *Bacillus cereus* TS1, 16S rDNA, Rice seed, Keratinase, PGPR.

Introduction

Microorganisms are an important component of the soil and play a key role in plant health and nutrition. Many soil bacteria can trigger plant growth through direct effects such as IAA production and phosphate solubilization. Plant growth promotion by these bacteria also contributes to indirect effects such as the biocontrol of soil borne fungal pathogens through siderophore mediated competition for iron, antibiosis, hydrolytic enzyme production or the induction of systemic resistance in the plant host (Lateef *et al*., 2010). Ammonia production by soil bacteria has been variably viewed, while it is considered effective from the biocontrol point of view (Li *et al*., 2009).

The heavy metals in general cannot be biologically changed to more or less toxic products and hence, persist in the environment. Moreover, the increasing concentrations of toxic heavy metals affect adversely both the plant growth promoting rhizobacteria and bio geochemical process mediated by

* Corresponding author: T. Sivakumar; e-mail: sivasadhana@yahoo.co.in

them. Further, the enhanced concentration of metals in soil can also have undesirable effects on plants. For instance, the accumulation of metals in plant organs to a undesired level show limiting effects on physiological processes such as photosynthesis and synthesis of chlorophyll pigments (Bibi and Hussain, 2005; Thirumalai arasu *et al*., 2009a) and also inactivate plant protein which subsequently reduce the crop yields severely. Metal showed toxicity towards rhizobial cells in sludge treated soils more than 10 years after addition of 250 mg Zn kg^{-1} (Chaudri *et al*., 2000). On the other hand the increasing concentration of metals in soil demonstrate a substantial reduction in N_2 fixation by white clover (*Vicifaba*) grown in soil irrigated with sludge (Assche and Clijsters, 1990; Broos *et al*., 2005).

Plant growth promoting rhizobacteria when applied to seeds or incorporated into soil reduce the toxicity of heavy metals and consequently enhance the growth and yield of plant. Further, the nodule bacteria can protect the plants against the toxic effects of nickel and zinc through adsorption or desorption mechanism (Glick *et al*., 1999). In addition, the plant growth promoting rhizobacteria also synthesize plant growth promoting substances (siderophore, indole acetic acid, hydrogen cyanide and ammonia), which augment the crop productivity (Rajkumar and Freitas, 2008). Moreover, these microorganisms stimulate the growth of host plants by fixation of atmospheric nitrogen and phosphate solubilization. This study was mainly focused on the assessment of *Bacillus cereus* TS1 for the activity of seed germination for rice seedling growth and nickel accumulation plant growth property was observed.

Materials and methods

Isolation of Bacillus cereus TS1 from the feather dumping site

In this study, *Bacillus cereus* TS1 was isolated from the feather dumping site. This isolate was identified by biochemical characters, carbohydrate characters, FAME analysis and 16S rDNA gene analysis. The 16S rRNA gene sequence of the isolate had been submitted to the Genbank under the accession number FJ377886 for *Bacillus cereus* TS1 (Sivakumar *et al*., 2011).

IAA Production

IAA was quantified by the method of Patten and Glick (2002). *Bacillus cereus* TS1 was cultured in flasks containing 10 ml of nutrient broth supplemented with tryptophan (L-Trp) 0.2 mM and incubated at room temperature (25 to 28°C) for 48 h. The cultures were then centrifuged for 15 min at 10000 rpm. Each 2 ml of the supernatant was mixed with 2 ml of

Salkowski's reagent (150 ml H_2SO_4, 250 ml distilled water, 7.5 ml $FeCl_3.6H_2O$ 0.5 M) and incubated at room temperature for 30 min. The presence of IAA was determined by the development of pink colour and the IAA concentration was measured spectroscopically at 520 nm and quantified in an IAA standard curve.

Hydrogen Cyanide (HCN) Production

Bacillus cereus TS1 was screened for the production of hydrogen cyanide by adapting the method of Lorck (1948). Nutrient broth was amended with glycine and bacteria were streaked on modified agar plate. Whatman filter paper No.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate. Plates were sealed with parafilm and incubated at 28°C for 4 days development of orange to red colour indicated the production of hydrogen cyanide.

Phosphate solubilization

Bacterial isolate was evaluated for the ability to solubilise inorganic phosphate in Pikovskaya's agar medium (HiMedia, Mumbai) containing calcium phosphate. A loopful of bacterial culture was placed on the plates and kept for incubation at 28°C for 7 days. The presence of clear zone around the bacterial colonies indicates the solubilization of phosphate (Ponmurugan Karuppiah and Shyamkumar, 2011).

Siderosphore production

Siderophore production was tested qualitatively using Chrome Azurol-S medium (CAS-medium) (Husen, 2003). *Bacillus cereus* TS1 was streaked on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation, and this test was done in two replications.

Seed germination

The ability of the selected isolate to promote seedling growth was examined according to the method of Rajkumar and Freitas (2008); Thirumalai arasu *et al.* (2009b). Rice seeds were surface sterilized with 0.1% $HgCl_2$ for 3 min and successively washed with sterile distilled water. Bacterial cells were washed twice with 100 mM $MgSO_4$ to remove spent medium and then suspended in 100 mM $MgSO_4$; Equal numbers of seed were incubated in

feather hydrolysates (0.15 OD at 600nm) or with 15 ml of 100 mM $MgSO_4$ for 1 h at room temperature. Fifty seeds of each treatment were placed in sterile 0.8% agar plates and kept at room temperature in dark. All the treatments were set up in triplicate value. Visual assessments of the seed germination were made daily up to 7 days.

Pot study for rice plant growth

Soil was sterilized by autoclaving at 15 lbs pressure at 121ºC for 15 mins. The seeds were surface sterilized and incubated for 1h at room temperature with 5ml of either 100 mM $MgSo_4$ (control) or *Bacillus cereus* TS1 bacterial suspension (0.02 OD). The sterilized soil was transferred to the pots ($3/4^{th}$). The seeds were sowed into the soil. Water was poured onto the pots regularly. The pot without culture was used as control. After 90 days of incubation, seedlings were harvested. Seed germination (%) shoot length (cm) and root length (cm) were determined and vigour index was calculated. Finally, the results were compared with control which was treated only with water and increased vigour index was also determined (Cunningham *et al*., 1995; Gholami *et al.,* 2009).

Vigour Index

Plant growth promoting properties was determined based on the vigour index and calculated as follows. Seed germination (%) – percentage of water soaked seeds germinated after 72 h of incubation at room temperature (Gholami *et al.,* 2009).

Vigour index = Root length + Shoot length × Seed Germination %

$$\text{Relative Vigour index (\%)} = \frac{\text{Vigour index of the isolates} - \text{Vigour index of the control}}{\text{Vigour index of the control}} \times 100$$

Influence of PGPB (Bacillus cereus TS1) on Ni uptake by rice seeds

Bacillus cereus TS1 was tested against various heavy metals namely cadmium, chromium, lead, nickel and zinc. *Bacillus cereus* TS1 was serially diluted using 25 mM Phosphate buffer and spread over Luria Bertani Medium (LB) amended with 50 mg of heavy metals. The plates were incubated at 37ºC for 48hrs. Among the various heavy metals used the isolate *Bacillus cereus* TS1 was resistant to nickel.

For nickel uptake pot experiments, the soil was collected from the Botanical garden, Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. The soil was sieved (2 mm) and sterilized by steaming (100ºC for 1h on three consecutive days). After sterilization the soil was amended with aqueous solution of $NiCl_2$ to achieve the final concentrations of 100, 200 or 300 mg Ni gm^1 and left in a greenhouse for 2 weeks period (for metal stabilization).

Rice seeds were inoculated with bacterial suspension (*Bacillus cereus* TS1) after adjusting OD to1.0 at 600nm. Seeds soaked in sterile water were used as control. The inoculated and non inoculated seeds were planted in plastic pot containing 1kg of soil. The plants were grown in a greenhouse at 25ºC and a 12/12 day/night regime. After 90 days the plants were carefully removed from the pots and the root surface was cleaned several times with distilled water. Growth parameters such as root length, shoot length, fresh weight and dry weight of the plants were measured (Rajkumar and Freitas, 2008).

Estimation of chlorophyll

To extract the total chlorophyll from leaves, fresh leaves were deveined and cut into small bits. From the pooled leaf bits, a sample of 100 mg was weighed. The leaf bits were homogenized in 100% acetone using a mortar and pestle. The homogenate was centrifuged at 4000 rpm for 5 mins at room temperature. Extraction with 100% acetone was repeated until the pellet becomes pale yellow or white in color. The supernatant was used for the estimation of photosynthetic pigments. The absorbance was measured at 662 nm, 645 nm and 470 nm for chlorophyll *a*, chlorophyll *b* and Carotenoids, respectively using ELICO SL 171 Spectrophotometer. The amount of chlorophyll *a, b* and total chlorophyll was calculated by using the formula of Wellburn and Lichtenthaler (1984).

Chlorophyll *a* (mg/L) $= 11.75 \times A_{662} - 2.35 \times A_{645}$
Chlorophyll *b* (mg/L) $= 18.61 \times A_{645} - 3.96 \times A_{662}$
Total Chlorophyll (mg/L) $= 7.79 \times A_{662} + 16.26 \times A_{645}$

Results

Measurement of IAA

Bacillus cereus TS1 isolate has the ability to produce IAA in various concentrations. The tested *Bacillus cereus* TS1 showed IAA production in culture supplemented with tryptophan (Trp). It was able to produce IAA in the highest level (10.21mg/L) in the culture supplemented with L-Trp (Table. 1).

Hydrogen cyanide mediated PGPR isolate

Bacillus cereus TS1 shows the level of PGP activities in relation to hydrogen cyanide (Table. 1).

Phosphate solubilisation

Isolate *Bacillus cereus* TS1 promoted rice seedling significantly and showed phosphate solubilization activity (Table. 1).

Siderophore production

CAS medium was used to investigate siderophore production by *Bacillus cereus* TS1. It is confirmed by the development of orange halos surrounding that colony (Table. 1).

Table 1. Indole acetic acid, hydrogen cyanide, phosphate solubilization and siderophore production by *Bacillus cereus* TS1

Isolate	Indole acetic acid production (mg/L)					Hydrogen cyanide production	Phosphate solubilization	Siderophore production
	20	40	60	80	100			
Bacillus cereus TS1	2.12	3.99	5.45	7.97	10.21	+++	+++	++

+ low colour intensity (or) zone formation ; ++ medium colour intensity (or) zone formation; +++ high colour intensity (or) zone formation.

Effect of feather hydrolysates on rice seed germination and rice plant growth

The feather hydrolysates obtained through keratinase of *Bacillus cereus* TS1 was sprayed on the rice plant. Rice seedling germination ability, root length and shoot length were evaluated (Table. 2 and 3; Fig. 1 and 2). Feather hydrolysates of *Bacillus cereus* TS1 keratinase promoted improvement in growth with increased vigor index of 66.26% compared with 0% control. Maximum increased values for *Bacillus cereus* TS1 shows vigor index in the percentage of above 100%. The seeds treated with feather hydrolysates of *Bacillus cereus* TS1 exhibited maximum shoot length (43cm) and root length (140cm) compared with untreated control shoot length is (35cm) and root length (95cm).

Table 2. Effect of *Bacillus cereus* TS1 on seed germination

Strains	RL	SL	Seed Germination (%)	VI	IVI (%)	RL		SL	
						FW	DW	FW	DW
Control	35	95	42.12 ± 0.25	5475.60	0	29.7 ± 0.14	32.0 ± 0.11	75.0 ± 0.12	29.3 ± 0.94
TS1	43	140	54.89 ± 0.47	9129.87	66.26	32.0 ± 0.54	39.2 ± 0.61	93.0 ± 0.54	31.2 ± 0.44

RL - Root length; SL - Shoot length; FW - Fresh weight; DW - Dry weight; VI - Vigor index; IVI - Increased vigor index; Values are the average of triplicate values with ± SE.

Table 3. Effect of *Bacillus cereus* TS1 on rice plant growth

Strains	RL	SL	Seed Germination (%)	VI	IVI (%)	RL		SL	
						FW	DW	FW	DW
Control	2.2	8.0	42.12 ± 0.8	429.62	0	5 ± 0.12	1 ± 0.17	12 ± 0.12	13 ± 0.30
TS1	3.8	13.0	54.17 ± 0.5	910.05	111.82	7 ± 0.45	2 ± 0.45	21 ± 0.46	18 ± 0.32

RL - Root length; SL - Shoot length; FW - Fresh weight; DW - Dry weight; VI - Vigor index; IVI - Increased vigor index; Values are the average of triplicate values with ± SE

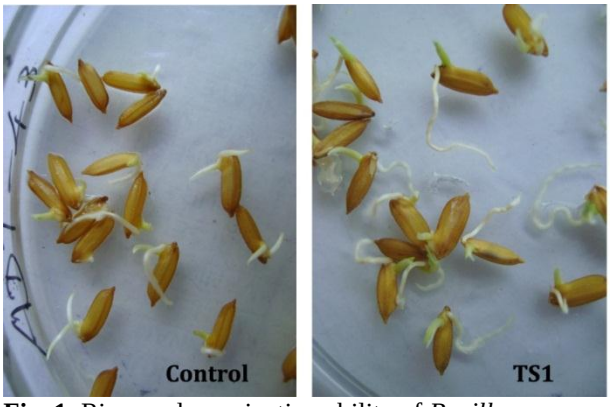

Fig. 1. Rice seed germination ability of *Bacillus cereus* TS1

Fig. 2. Rice Plant growth activity of *Bacillus cereus* TS1 after 90 days

Effect of Nickel on growth of rice seed

In our study, the significant increase in the fresh and dry biomass of the crop plants treated with *B. cereus* TS1 suggests the successful colonization and subsequent plant growth promoting potentiality of the strain in rice plants (Fig. 3). The best performer in the respective rice plant growth promotion potential was recorded for *B. cereus* TS1 (Table. 4).

Table 4. Influence of Nickel mobilizing bacteria (*Bacillus cereus* TS1) on the fresh weight and dry weight of rice seeds; Nickel uptake shoot and root dry mass and weight (mg/kg) on rice plants

Characters	**Rice seedling growth**	
Shoot length	Control	31.70 ± 2.02
	TS1	36.6 ± 1.65
Root length	Control	70.23 ± 1.96
	TS1	112.86 ± 2.20
Total chlorophyll content	Control	0.53 ± 0.01
	TS1	0.77 ± 0.003
FW	Control	29.8 ± 0.5
	TS1	35.0 ± 0.7
DW	Control	14.7 ± 0.3
	TS1	19.2 ± 0.2
Shoot dry mass and weight (Nickel concentration (mg/kg))	Control	28.40 ± 3.02
	TS1	38.6 ± 3.65
Root dry mass and weight (Nickel concentration (mg/kg))	Control	120.23 ± 2.96
	TS1	250.36 ± 4.20

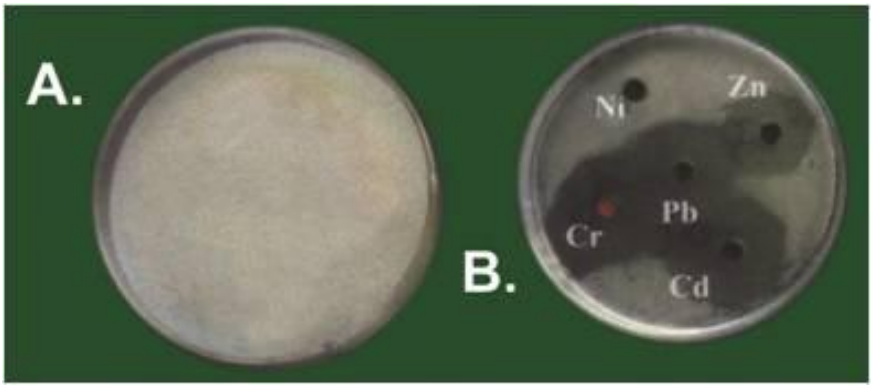

Fig. 3. Metal resistant of TS1 for nickel (A. Control, B. *Bacillus cereus* TS1).

Discussion

In this study, we obtain sufficient information regarding the diversity and plant growth promoting activity of *Bacillus cereus* TS1 indigenously isolated from Sivakasi, Tamilnadu. The ability of the isolates to increase plant growth in germinating seed bioassay is highly related to the IAA production, which was produced by *Bacillus cereus* TS1 isolate. Varying results of germinating seed assay had also pointed out that, there was complex interaction between bacterial IAA and seedlings; therefore it caused different responses of plant growth. Yet,

there is stimulation of bacterial IAA to the development of the host plant root system (Patten and Glick, 2002). In addition, Patten and Glick (2002) also reported that, low levels of IAA can stimulate root elongation, while high levels of bacterial IAA, stimulates the formation of lateral and adventitious roots. In this study, IAA produced by *Bacillus cereus* TS1, promotes seed growth.

Hydrogen cyanide production by Azotobacter isolates were about 60% for the inhibition of phytopathogens in the soil. Most of the *Bacillus* sp. isolated (BA1, BA3, BA4, BA6, BA7 and BA8) from soils of vegetable plants are producing HCN as well as siderophore and act as potent antifungal agent. Synergistic interaction of these two with other metabolites may further function as stress factors including local and systematic host resistance that led for the suppression of the root pathogens (Rekha *et al*., 2010).

Siderophore is one of the biocontrol mechanisms belonging to PGPR groups, including *Bacillus* sp. under iron limiting condition. PGPR produces a range of siderophore, which have a very high affinity for iron. Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi (Whipps, 2001). The present study reveals that the *Bacillus cereus* TS1 was exhibiting high level of HCN as well as siderophore, hence it may poses antimicrobial activity which may inhibit phytopathogenic microbes present in the soil.

The ability of several isolates to solubilize tri calcium phosphate *in vitro* shows the possible application of the isolates in crop fields. Rodriguez and Fraga (1999) demonstrated that, *Pseudomonas* and other phosphate solubilizing bacteria (PSB) like *Bacillus* sp were capable of increasing the availability of phosphorus in soil. Our study revealed that, *Bacillus cereus* TS1 significantly promoted rice seedling, were able to solubilize phosphate.

The feather degrading capability of the keratinolytic bacteria accelerate the composting of chicken feather waste and convert this organic material into nitrogen fertilizers. In our study, when compared to the control the seed treated with feather hydrolysates of *Bacillus cereus* TS1 showed above 100% increased vigour index due to secretion of some plant growth hormones such as IAA and synthesis of some beneficial amino acids. The protein rich concentrate feather meal generated for poultry feed can also be applied for organic farming as a semi slow release nitrogen fertilizers. Organic farming relies on the use of nitrogen rich organic amendments that serve the dual purpose of improving plant growth and intensifying microbial activity in soil. Traditionally, guano has been widely used as a fertilizer for organic farming (Hadas and Kautsky, 1994). However, owing to high expenses, there is a need to search for more suitable alternatives. Feather meal is being a nitrogen rich (15% N), in expensive and readily available source serves as a potential substitute to guano.

It not only supplies nitrogen to plants and promotes microbial activity, but also structures the soil and increases water retention capacity. The microbial hydrolyzed feather meal can further edge over the steamed meal as fertilizer due to its high nutritive value, easy production and economic feasibility.

Grazziontin *et al*. (2007) have demonstrated the strains *Vibrio* sp. Kr2 produced hydrolysates rich in soluble protein (2.5g/l) on growing in 40, 60 or 80g/L feathers. Similarly, the germination percentage of ryegrass increased with increasing amount of the added alkaline hydrolysate of sheep's wool waste reaching values of 80% on day 15 (10-fold higher than control sample) (Nustovora *et al*., 2005) would establish an economical and environmental safe method of recycling these organic materials into high nitrogen fertilizers. Hence the keratinase of *Bacillus cereus* TS1 can be exploited for agricultural applications. Microorganisms are important components of the soil and play a key role in plant health and nutrition. Many soil microorganisms can trigger plant growth through direct effects such as IAA production and phosphate solubilization. Jeong *et al*. (2010) reported that the *Stenotrophomonas maltophilia* R13 produced IAA, ammonia and several hydrolytic enzymes such as pectinases, proteases and lipase.

Maximum plant growth promoting effect was observed in *Bacillus cereus* TS1 which enhances the shoot length, root length, chlorophyll content, fresh weight and dry weight by 36.70, 112.86, 0.77, 35.8 and 19.2 respectively.

At a concentration of 10 mg nickel/gm of soil the highest effect was found for *Bacillus cereus* TS1 which enhanced shoot length, root length, chlorophyll content, fresh weight and dry weight. The bacteria inoculated and non-inoculated plants were subjected to three levels of Ni in soil for 45 day responded differently in terms of plant growth. In the absence of Ni, inoculation of *Pseudonmonas* sp. Ps29C or *B. megaterium* Bm4C showed an increase in shoot length, fresh and dry weight of plant. Growth promotion in plants in due to the presence of PGPB in the soil, a common feature, and has been observed in many plant species (Balasubramanian and Pugalenthi, 2000; Wu *et al*., 2007).

The non-inoculated plants exposed to different concentrations of Ni, showed a marked inhibition in the growth. In general, with the increase in concentration of Ni progressive decrease in plant fresh and dry weight was observed. At a concentration of 100 mg Ni kg^{-1} soil, the percent decrease was 6 for fresh weight and 8 for dry weight; for 200 mg Ni, 7 and 10%; and for 300 mg Ni, 17 and 18%, respectively. Growth reduction at higher concentration may be due to the toxic effects of Ni on plants. Moftah (2000) and Panwar *et al*. (2002) also reported similar results in *B. juncea* with increasing Ni content of soil (0-80 mg kg^{-1}).

Wang (1989) stated that the plants inoculated with *Pseudomonas* sp. Ps29C or *B. megaterium* Bm4C exhibited an increase in shoot length, plant fresh and dry weight in the presence of Ni. At a concentration of 100 mg Ni kg^{-1} soil, the highest effect was found for *B. megaterium* Bm4C, which enhances the shoot length, fresh weight and dry weight by 33, 74 and 81%, respectively. Similarly, at a concentration of 200 mg Ni, the percent increase was 45 for shoot length, 70 for fresh weight and 25 for dry weight; for 300 mg Ni, 31, 73 and 40%, respectively. The results obtained here clearly indicate that inoculation of *Pseudomonas* sp. Ps29C and *B. megaterium* Bm4C not only protects Indian mustard from Ni toxicity but also promotes the plant growth. Mamaril *et al*. (1997 and Ying (2009) has reported that the efficiency of revegetation and phytoremediation of heavy metal-contaminated sites is closely related to the presence of metal resistant microbial populations in the soil, which likely conferred a better nutritional assimilation and protection effect on plants.

Conclusion

The data gathered in this study gives the hints about the phosphatase, IAA, HCN and siderophore producing ability of the isolate *Bacillus cereus* TS1. As well as the strain can be used as a PGPR, in nickel contaminated soil.

Acknowledgement

The authors would like to acknowledge the Department of Microbiology in Ayya Nadar Janaki Ammal College, Sivakasi for providing the facility to carry out this work successfully.

References

Assche, F.V. and Clijsters, H. (1990). Effects of metals on enzyme activity in plants. Plant Cell. Environ 13:195–206.

Balasubramanian, S. and Pugalenthi, V. (2000). A comparative study of the determination of sulphide in tannery waste water by ion selective electrode (ISE) and iodimetry. Water Res 34:4201-4206.

Bibi, M. and Hussain, M. (2005). Effect of copper and lead on photosynthesis and plant pigments in black gram (*Vigna mungo* L.). Bull. Environ. Contam. Toxicol 74:1126–1133.

Broos, K., Beyens, H. and Smolders, E. (2005). Survival of rhizobia in soil is sensitive to elevated zinc in the absence of the host plant. Soil Biol. Biochem 37:573–579.

Chaudri, A.M., Allain, C.M., Barbosa J.V.L., Nicholson, F.A., Chambers, B.J. and McGrath, S.P. (2000). A study of the impacts of Zn and Cu on two rhizoidal species in soils of a long term experiment. Plant Soil 22:167–179.

Cunningham, S.D., Berri, W.R. and Haung, J.W. (1995). Phytoremediation of contaminated soil. Trends Biotecl 134:393–397.

Gholami, A., Shahasavani, S. and Nezarat, S. (2009). The effect of plant growth promoting rhiobacteeria on germination, seedling growth and yield of maize. World Acad Sci, Eng and Tech. 49:56–59.

Glick, B.R., Patten, C.L., Holguin, G. and Penrose, G.M. (1999). Biochemical and Genetic mechanisms used by Plant Growth Promoting Bacteria. Imperial College Press, London. pp. 510-516.

Grazziotin, A., Pimentel, F.A., Sangali, S., DeJong, E.V. and Brandelli, A. (2007). Production of feather protein hydrolysates by keratinolytic bacterium *Vibrio* sp. kr2. Bio Tech. 98: 3172-3175.

Hadas, A. and Kautsky, L. (1994). Feather meal, a semi slow release nitrogen fertilizers for organic farming. Ferti Res. 38:165-170.

Husen, E. (2003). Screening of soil bacteria for plant growth promotion activities *in vitro*. Indo. J. of Agr. Sci. 4(1):27-31.

Jeong, J.H., Milee, O., Jeon, Y.D., Do, J., Ri Lee, N., Yeol, L. and Chung, H. (2010). Production of keratinolytic enzyme by isolated feather degrading *Stenotrophomonas maltophibra* for plant growth promoting activity. Pro. Biochem 45:1738-1745.

Lateef, A., Oloke, J.K., Kana, E.B.G., Sobo, B.O., Ajao, S.O. and Bello, B.Y. (2010). Keratinolytic activities of a new feather degrading isolate of *Bacillus cereus* ILAU 08 isolated from Nigerian soil. Int. Biodet. Biodeg 164:478-485.

Li, S., He, B., Bai, Z. and Ouyang, P. (2009). A novel organic solvent stable alkaline protease from organic solvent tolerant *Bacillus licheniformis* YP1A. J. Mol. Biol. Enz 56: 85–88.

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. Plant Physio. 1:142–146.

Mamaril, J.C., Paner, E.T. and Alpante, B.M. (1997). Biosorption and desorption studies of chromium (iii) by free and immobilized *Rhizobium* (BJVr 12) cell biomass. Biodegradation 8:275–285.

Moftah, A.E. (2000). Physiological response of lead polluted tomato an egg plant to the antioxidant ethylene diurea. Agri. Res. 25:933–955.

Nustovora, M., Braikova, D., Gousterova, A., Tonkova, E.V. and Nedkov, P. (2005). Chemical, microbiological and plant analysis of soil fertilized with alkaline hydrolysates of sheep's wool wastes. World J. Micro. Biotech. 5:9045-9049

Panwar, B.S., Ahmad, K.S. and Mittal, S.B. (2002). Phytoremediation of nickel contaminated soils by *Brasssica* sp. Environ. Sustain. 4:1–6.

Patten, C.L. and Glick, B.R. (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. App. and Environ. Micro. 68:3795-3801.

Ponmurugan, K. and Shyamkumar, R. (2011). Exploring the Potential of Chromium Reducing *Bacillus* sp. and there Plant Growth Promoting Activities. J. of Micro. Res. 1(1):17-23.

Rajkumar, M. and Freitas, H. (2008). Effects of inoculation of plant growth promoting bacteria on Ni uptake by Indian mustard. Biores. Tech. 99:3491–3498.

Rekha, V., Ahmed John, S. and Shankar, T. (2010). Antibacterial activity of *Pseudomonas fluorescens* isolated from Rhizosphere soil. Int. J of Biol. Tech. 1(3):10-14.

Rodriguez, H. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 17:319–339.

Sivakumar, T., Ramasubramanian, V., Shankar, T., Vijayabaskar, P. and Anandapandian, K.T.K. (2011). Screening of keratinolytic bacteria *Bacillus cereus* from the feather dumping soil of sivakasi. J. of Basic and App. Bio. 5(1&2):305-314.

Thirumalai arasu, V., Sivakumar, T., Ramasubramanian,V., Nalini, K. and Vanathi, R. (2009a). The potential application of keratinase from *Bacillus* sp. as a laundry detergent and feed additives. J. Adv. Biotech. 4:21-25.

Thirumalai arasu, V., Sivakumar, T., Ramasubramanian,V., Nalini, K. and Kiruthiga, R. (2009b). The potential application of keratinase from *Bacillus* sp. as plant growth promoters . J. Pure Appl. Micro 3(2):583-596.

Wang, P.C., Mori, T., Komori, K., Sasatsu, M., Toda, K and Ohtake, H. (1989). Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. App. Environ. Micro. 55:1665–1669.

Wellburn, A.R. and Lichtenthaler, H. (1984). In: Advances in photosynthesis Research (ed.Sybesma) Martinus Nijhoff, Co., The Hague, Vol. II, and pp:9-12.

Whipps, J.M. (2001). Microbial interactions and biocontrol in therhizosphere. J. Exper. Bot 52: 487-511.

Wu, Q.L., Chen, T., Gan Y., Chen, X. and Zhao, X.M. (2007). Optimization of riboflavin production by recombinant *Bacillus sustilis* RH44 using statistical designs. App. Micro. Biotech. 76:783-794.

Ying, M., Mani, R. and Helena, F. (2009). Isolation and characterization of Ni mobilizing PGPB from serpentine soils and their potential in promoting plant growth and Ni accumulation by *Brassica* sp. Chemsphere 75:719-725.

(Received 20 April 2012; accepted 30 October 2012)