Research Article

Journal of Plantation Crops, 2014, 42(3): 316-322

Studies on nutrient solubilization, biocontrol and plant growth promoting traits of *Burkholderia cepacia* from tea soil

B. Bagyalakshmi*, P. Ponmurugan and A. Balamurugan[1]

Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode - 637 215, Namakkal District, Tamil Nadu, India

[1]Division of Plant Pathology, UPASI Tea Research Institute, Valparai,Coimbatore, Tamil Nadu, India

(Manuscript Received: 01-02-13, Revised: 15-05-14, Accepted: 19-06-14)

Abstract

A study was undertaken to isolate a novel indigenous bacterial strain namely *Burkholderia cepacia* from tea soils to solubilize potassium (K) and phosphorus (P) sources respectively. The isolated strain was screened based on its solubilization potential in both broth and agarized medium amended with various K and P sources. Plant growth promoting traits and biocontrol activity of the purified strain against tea pathogens such as *Pestalotiopsis theae, Glomerella cingulata, Poria hypolateritia, Phomopsis theae and Hypoxylon serpens* were studied. Results revealed a significant solubilizing zone in agar medium blended with muriate of potash (MOP) (2.0 cm), sulphate of potash (SOP) (1.2 cm), rock phosphate (1.6 cm) and single super phosphate (0.8 cm). The release of available K was quantified in liquid medium supplemented with MOP and was found to be higher (41.5 mg L^{-1}) than SOP. Among different P sources, rock phosphate (35.2 mg L^{-1}) showed higher solubilization than single super phosphate (30.2 mg L^{-1}) by the test organism on 5th day of incubation. *B. cepacia* was found to produce a large amount of bioactive compounds like siderophore (12.3 µg mL^{-1}), IAA (263.3 µg mL^{-1}) and GA_3 (14.9 µg mL^{-1}) including exo-polysaccharides (46.8 ppm). The test organism also showed a remarkable biocontrol activity against *P. theae* (52.5%), *G. cingulata* (42.5%), *H. serpens* (47.5%), *Phomopsis theae* (32.7%) and *P. hypolateritia* (30.9%). The secondary metabolites production by an efficient strain *B. cepacia* revealed that the strain could produce a wide range of volatile compounds.

Keywords: Antagonism, phosphate solubilizer, potassium solubilizing bacteria, tea soil

Introduction

Potassium (K) and phosphorus (P) are major nutrients and play important role in plant growth. A significant amount of these nutrients are present in the soil as unavailable forms and could be converted into soluble/available forms during plant growth through microbial activity. Usage of mineral solubilizing bacteria as biofertilizer *viz*., potassium solubilizing bacteria (KSB) and phosphate solubilizing bacteria (PSB) can be useful in increasing the availability of these nutrients for plant growth by solubilisation process (Sheng *et al.,* 2003). Microbial solubilization of nutrients is the only way to increase the available K and P for crop development (Wu *et al.,* 2005).

In tea plantation fertilizers such as muriate of potash (MOP), sulphate of potash (SOP) and rock phosphate were being applied continuously which lead to the deterioration of soil health and fertility. Sheng and Haung (2002) reported the reasons for K deficiency in soil utilization by crops, runoff, leaching and soil erosion. The conversion of different mineral forms to available K could be through high acidification, chelation and exchange reactions. The solubilisation of K by using *Bacillus edaphicus* increased K uptake in wheat, cotton and grape plants. K solubilizing bacteria namely, *B. mucilagenosus* was isolated from soils (Xiufang *et al.,* 2006). The potential of PSB *B. megaterium* and KSB, *B. muciloginosus* was evaluated in soils planted with egg plant by Han and Lee (2006).

* *Corresponding Author: florabalan@gmail.com*

Similarly, *Frateuria aurantia* belonging to the family Pseudomonaceae obtained from the agricultural soils of Tamil Nadu had been used successfully in many crops (Ramarethinam and Chandra, 2006). Co-inoculation of PSB and KSB increased the mineral uptake and enhanced the growth of pepper and cucumber (Han and Lee, 2006).

Both PSB and KSB possess the ability to solubilize different inorganic P and K respectively, thereby making it available to plants by producing certain organic acids, vitamins and growth promoting substances like IAA and GA_3 which help to promote growth of the plants (Ponmurugan and Gopi, 2006). Different bacterial groups of *Pseudomonas, Agrobacterium*, *Bacillus, Rhizobium*, *Flavobacterium* and *Burkholderia* have been studied for their ability to solubilize different P sources (Goldstein, 1994). Usage of these organisms as biofertilizers or biocontrol agents in agriculture has been a focus in recent research. Application of this indigenous microbial inoculum as biofertilizers in tea plantation resulted in higher productivity. A study was undertaken to isolate and characterize indigenous KSB and PSB from tea soil, to evaluate their efficiency in solubilization of different K and P sources and plant growth promoting characters for field application in tea plantation.

Materials and methods

Isolation of potassium and phosphate solubilizing bacteria from tea soils

Soil samples were collected (0-9 inch depth) by using augor from the tea garden planted with clone UPASI-9 (Murugali Estate – Parry Agro Industries, Valparai) during pre-monsoon season in the year of 2010. KSB was isolated in Aleksandrov medium (Aleksandrov *et al.,* 1967) incorporated with MOP and SOP by following dilution plate technique. KSB colonies were identified by the formation of clear/ transparent zone around the bacterial colonies due to the solubilization of K source. For P solubilizing study, was done using Pikovskaya's medium (Pikovskaya, 1948) supplemented with rock phosphate and single super phosphate. A clear zone around the bacterial colony indicated solubilization of P sources and was designated as VKSR10 and selected for quantitative study in respective liquid medium. The entire work was carried out in department of Biotechnology, K.S.R. College of Technology, Tiruchengode, Namakkal district of Tamilnadu.

Quantitative assay of potassium and phosphate solubilization

Solubilizing efficiency of the selected strain (VKSR10) was carried out using Aleksandrov broth supplemented with 0.2 per cent of MOP and SOP. The culture was withdrawn at 3rd, 5th and 7th day of incubation and centrifuged to collect supernatant. This cell free culture filtrate solution was used to determine the pH and available K content was measured using flame photometer. Similarly, the available P content in the medium supplemented with P sources, supernatant was taken and estimated by following the method of Natarajan and Buvana (2000).

Characterization of selected strain - VKSR10

The bacterial strain showing higher solubilization of both K and P sources was selected and identified. Generic level of identification was carried out by following morphological, physiological and biochemical traits by adopting various polyphasic taxonomic approaches (Collins and Lyne, 1980). Molecular identification of the efficient strain was carried out (Sambrook *et al.,* 1989).

Plant growth promoting traits of VKSR10

The production of plant growth hormones such as indole acetic acid (IAA) and gibberellins (GA_3) by VKSR10 strain was estimated in the basal medium supplemented with commercial IAA and GA_3 substrates by the method of Tien *et al.* (1979) and Mahadevan and Sridhar (1982) respectively. The production of siderophores, exopolysaccharide and hydrogen cyanide was estimated by following the method of Cappuccino and Sherman (1992).

Biocontrol activity and production of cell wall degrading enzymes in VKSR 10

The biocontrol activity of strain was performed by following dual inoculation technique using potato dextrose medium (Sakthivel and Gnanamanickam, 1987) against tea foliar pathogens, *viz., Pestalotiopsis theae, Glomerella*

cingulata; stem pathogens *viz., Phomopsis theae, Hypoxylon serpens* and root pathogens *viz., Poria hypolateritia and Botryodiplodia theobromae*. About 5 mm diameter of fungal mycelia plug obtained from peripheral region of 7 days old culture of pathogens and one day old KSB strain were inoculated in opposite end on a Petri dish and incubated at 27±2 ºC for 2-3 days. The inhibition of pathogen growth was calculated over the untreated control plate.

For the production of cell wall degrading enzyme study, the mineral salts medium (Sakthivel and Gnanamanickam, 1987) was incorporated with various enzyme substrate sources such as chitin, pectin, carboxy methyl cellulose and were individually added at 0.1% concentration in the basal medium. The strain was inoculated by simple streaking on the medium with suitable substrates and incubated at room temperature for 72 hours to observe their growth. Utilization of the substrates by KSB strain was designated as positive (+) and negative (-).

Secondary metabolites of VKSR10 was analysed through gas chromatography-mass spectrometry (GC-MS) method. The potent KSB strain (VKSR10) was generically identified as *Burkholderia cepacia* through molecular tools.

Results and discussion

A total of 68 isolates were obtained from rhizosphere soils, in which about 12 were found to be efficient in terms of solubilization potential in the basal medium containing K and P sources. Among 12 isolates, the most efficient one was capable of solubilizing both minerals which had been selected and designated as VKSR10 for further studies.

The ability of *B. cepacia* to solubilize K and P were measured qualitatively and quantitatively in the basal medium. The zone produced by *B. cepacia* on Aleksandrov medium ranged between 1.0 and 1.6 cm (Table 1). The maximum solubilization of K was observed by an isolate with MOP source which measured about 1.6 cm in diameter. The results of P solubilization ability of the strain revealed that it produced higher solubilisation zone with rock phosphate (1.2 cm) than single super phosphate (0.8 cm) (Fig. 1a and 1b) among the two phosphate sources tested.

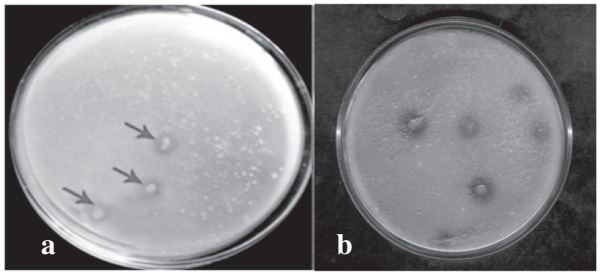

Fig. 1. Growth of bacterial strain (VKSR10) in (a) Aleksandrov agar medium and (b) Pikovskaya's agar medium. Arrows indicate the solubilisation zone around the bacterial colony

Further, K solubilizing efficiency of the isolate in Aleksandrov broth revealed that it readily solubilised MOP at faster and higher rate compared with SOP on 5th day of incubation period (41.5 mg L^{-1}) (Table 1). In case of pH, maximum solubilization of K content occurred in the basal medium and resulted in a pH of 4.6. There was a drop in pH of the broth medium from 6.8 to 4.6. A lesser solubilization of K was observed significantly in the basal medium incorporated with SOP (28.4 mg L^{-1}). The solubilisation process of minerals may be due to the production of various organic acids such as acetic, formic, gluconic, oxalic and succinic acids (Lal, 2002). Adeleke *et al.* (2010)

Table 1. Nutrient solubilisation efficiency of strain, VKSR10

Substrates used	Diameter of solubilization zone in (cm)	pH change in the medium	Phosphorus content (mg L^{-1})	Potassium content (mg L^{-1})
TCP	0.8±0.1	5.2±0.2	24.3±1.6	-
RP	1.2±0.2	4.9±0.2	35.3±1.2	-
MOP	1.6±0.2	4.6±0.1	-	41.5±2.2
SOP	1.0±0.1	5.0±0.2	-	28.4± 1.7

Values are mean of three replicates followed by standard error
TCP- tri-calcium phosphate; RP- rock phosphate; MOP- muriate of potash; SOP- sulphate of potash

have reported the ability of ectomycorhizal fungi in mobilization of P and K sources from insoluble ore. Greater release of K from muscovite has been recorded in *B. muciloginosus* (Sugumaran and Janarthanam, 2007). In the present study, all the isolated strains showed a change in pH towards acidic range due to secretion of certain organic acids by the organisms involved in the solubilization of K and/or P in turn reduced the pH of the medium.

The VKSR10 strain was further screened for its P solubilisation efficiency and release of available P in the basal medium while it was incorporated with RP and SSP individually (Table 1). The bacteria were able to solubilize the insoluble P into soluble form. The strain was found to solubilize and release the maximum P into the basal medium (35.3 ppm) when RP was used. A least P solubilization was found with SSP (24.3 ppm). A reduction in pH from 6.8 to 4.9 which might be due to the production of organic acids. Rhizosphere bacteria are capable of solubilizing inorganic P in soil environment by improving solubilization of unavailable forms of P present in soil by producing phosphatase enzyme. The results of the present study were concordance with the findings of Hu *et al.,* (2006).

Identification of VKSR10 strain

The selected strain (VKSR10) was characterized by following the method of Bergey's manual. The results of various biochemical tests are summarized in Table 2. The strain showed the characteristics features of *Burkholderia cepacia.* The strain was gram negative rod and motile in nature. Presence of endospores and capsule were not observed. The cells were capable of producing variety of enzymes such as oxidase, catalase, urease and the substrates like starch, casein and gelatine were also hydrolyzed. Release of acid and gas from glucose and nitrate was reduced by two cultures to the maximum extent. Further, the cultures failed to produce fluorescent pigments but were capable of producing hydrogen cyanide and siderophores. They solubilized insoluble form of P and K sources efficiently and utilized L-arabinose, D-xylose and D-mannitol.

Table 2. Characterization of efficient nutrient solubilizing strain (VKSR10)

Character traits	Status	Character traits	Status
Gram's staining	-ve	Nitrate reduction	+
Cell shape	rod	Casein hydrolysis	-
Motility test	+	Lipase production	-
Spore formation	-	Urease production	+
Capsule formation	-	Citrate utilization	+
Cytochrome oxidase	+	Indole production	+
Catalase test	+	Gas from glucose	+
Fluorescent pigment	-	Acid from glucose	+
Methyl red	-	L-Arabinose	+
Voges Proskauer	-	D-Xylose	+
Starch hydrolysis	+	D-Mannitol	-
Gelatin hydrolysis	+	Phosphate solubilisation	+
Arginine dihydrolysis	-	HCN production	+

+ Positive; - Negative

At molecular basis of identification, PCR amplification with genomic DNA of VKSR10 strain was carried out by using 16S rDNA as primer and yielded a fragment of 1500 bp length. The amplified and purified PCR products were subjected to BLAST analysis *via*., blast search through GenBank (http://www.ncbi.nlm.nih.gov). The results indicated that sequence identity of 99 per cent similarity to uncultured strain. From the above results, the VKSR10 strain was identified as *B. cepacia.* The sequence was submitted to NCBI (JQ610568).

Plant growth promoting traits and biocontrol activity of VKSR10

The quantification of plant growth hormones such as IAA and GA_3 produced by VKSR10 strain was estimated. The bacteria produced IAA higher

Table 3. Production of plant growth promoting traits and cell wall degrading enzymes by VKSR10

Plant growth promoting traits	Quantification
Siderophore (µmole mL^{-1})	22.4±1.3
HCN production	++
IAA (µg mL^{-1})	186.4±11.3
GA_3 (µg mL^{-1})	12.9±0.5
Exopolysaccharides (ppm)	46.8 ±1.9
Chitinase	++
Pectinase	++
Cellulase	++

Values are mean of three replicates with standard error
++ : status of high production

(186.4 µg L^{-1}) and less of GA_3 (12.9 µg L^{-1}) (Table 3). Another *in vitro* experiment was undertaken to study the production of biocontrol substances by VKSR 10 such as siderophore, hydrogen cyanide (HCN) and exopolysaccharides (EPS). The strain produced siderophore and EPS at maximum quantity of 22.4 µmoles mL^{-1} and 46.8 ppm, respectively (Table 3).

Table 4. Biocontrol activity of VKSR10 against tea pathogens

Tea pathogens	Zone of inhibition (cm)	Inhibition (%)
Pestalotiopsis theae	1.9 ±0.2	52.5
Glomerella cingulata	1.5 ±0.2	42.5
Hypoxylon serpens	0.8 ±0.1	47.5
Phomopsis theae	1.1 ±0.1	32.7
Poria hypolateritia	1.9 ±0.1	30.9
Botryodiplodia theobromae	1.7 ±0.2	42.1

When studying the biocontrol potential of tested bacteria, an array of cell wall degrading enzymes was produced in the basal medium when incorporated with respective substrates sources. Results revealed that the strain was capable of producing cellulase, pectinase and chitinase by utilization of their substrate sources (Table 3). Chitinase production in microorganism was reported to act as good antagonistic agent reported by Jha and Mathur (1993) and they supported the present biocontrol property of the strain VKSR10.

An *in vitro* experiment was performed to find out the antagonistic potential of bacterial isolate against certain tea pathogens. The results revealed that *B. cepacia* significantly antagonised the tested tea fungal pathogens (Table 4) and higher antagonism was recorded against *P. hypolateritia* (1.9 cm) followed by *B. theobromae* (1.7 cm). Similar biocontrol

Table 5. Spectral analysis of GC-MS data of secondary metabolites produced by *B. cepacia* (VKSR10 strain)

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Nature of compound	Activity
2.9	1-Octanol, 3,7-dimethyl-, (S)	$C_{10}H_{22}O$	158	27.9	Alcoholic compound	Antimicrobial
3.7	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)- [Camphor, (1S)-]	$C_{10}H_{16}O$	152	2.1	Monoterpene oxide	Antiacene, Analgesic, Anesthetic Antiseptic, Antispasmodic, Cancer preventive, Fungicide, Herbicide, Nematicide Stimulant, Insect repellent, Insectifuge, Decongestant, Emetic, Pesticide, Antifeedant
4.5	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	134	1.8	Aldehyde compound	Anesthetic, Antibacterial, Anticancer, Antiseptic Antitumor, Antispasmodic, Immunostimulant Insecticide, Insectifuge, Nematicide Pesticide, Sedative, Termiticide Tyrosinase inhibitor
5.5	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	172	4.4	Alcoholic compound	Antimicrobial
8.1	1-Nitrododecane	$C_{12}H_{25}NO_2$	215	0.6	Nitrogen compound	Antimicrobial
8.3	1-Pentanol, 4-methyl-2-propyl-	$C_9H_{20}O$	144	0.6	Alcoholic compound	Antimicrobial
10.3	Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$	234	2.4	Aromatic acid ester	Antimicrobial, Preservative
20.8	1,2-Benzene-dicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	0.5	Plasticizer compound	Antimicrobial, Antifouling

activity was observed by fluorescent Pseudomonads against multiple pathogens in tea (Muralidharan *et al.,* 2004). By which, the strain proved its biocontrol potential against tea fungal pathogens and further, the results revealed that the native strain VKSR10 imparted inhibition against foliar, stem and root pathogens of tea.

KSB strain was found to produce siderophores, exopolysaccharide and HCN. Siderophores play a vital role in the suppression of plant pathogens by chelation of ferric iron, thereby creating crisis of iron element to competitive partner (Loper and Henckels, 1999). Similar reports on production of siderophores, antifungal volatiles and antimicrobial metabolites by antagonistic fluorescent Pseudomonads. The observation on the production of polysaccharides by KSB had been reported by several researchers (Sheng and He, 2006). The bacterial action on the formation of mucilagenosus capsule consisting of exopolysaccharide and organic acids were reported by Vijayabaskar *et al.,* (2011).

Analysis of secondary metabolites produced by KSB strain by using GC-MS

The secondary metabolites production by the strain VKSR10 was analysed and data of activity compounds are shown in Table 5. The results revealed that bacteria could produce a wide range of volatile compounds and they were identified based on their peak area, molecular weight and Rt values (retention time) in the spectral diagram. The compounds were further compared with the mass spectra details from the compound library which indicated that a total of about 18 compounds. Those compounds belonged to aldehyde, esters, alcohols ketone, monoterpene oxide alkane and plasticizer. The maximum peak of 3-ethyl-3-methylheptane was observed (36.8) followed by 1-Octanol, 3,7-dimethyl,(S) (27.9) and 1-Undecene, 7-methyl (7.1). The minimum peak was observed with butanoic acid and 2-propenyl ester (0.4) in the spectral analysis. These identified metabolites from VKSR10 strain may provide information on the mechanism for solubilization of mineral sources, insecticidal and antimicrobial activity. Similarly, the antifungal compounds were identified from *Stenotrophomonas rhizophila* in relation to biocontrol activity by Sheng and He (2006). *Bacillus* spp. showing antifungal activity and antagonistic activity due to siderophores, antifungal volatiles and antimicrobial metabolites as mechanisms of biocontrol against a large number of pathogens has been reported. (Chakraborty *et al.,* 2006; Vidhya *et al.,* 2012).

Conclusions

The present study indicated that the *B. cepacia* (VKSR10) strain isolated from tea rhizosphere was found to produce various organic acids and solubilise insoluble forms of minerals and make them available to tea crop then they act as potential antagonists against tea pathogens thus proving the production of secondary metabolites, and cell wall degrading enzymes that has the potential to control variety of pathogens. Based on these results, it can be further inferred that *B. cepacia* can be used as a soil inoculant to prevent the growth of soil-borne pathogens in tea soils. The strain has capability to promote plant growth by production of certain phytohormones too. So, this *in vitro* observation on solubilization of potassium and phosphate mineral sources by this strain of bacteria indicated that they could be used as biofertilizers for reduction in use of inorganic fertilizers by minimizing the leaching and fixation of the elements in the tea soils, improvement of soil nutrients and minimizing the harmful pathogens.

Acknowledgements

The authors are thankful to the Principal and the Management, K.S.R. Educational Trust, Tiruchengode, Tamil Nadu for providing necessary facilities and constant encouragement and Dr. P. Mohankumar, The Director, UPASI TRF, TRI Valparai for technical support to carry out this study.

References

Adeleke, R.A., Cloete, T.E., Bertr, A. and Khasa, D.P. 2010. Mobilization of potassium and phosphorus from iron ore by ectomycorrhizal fungi. *World Journal of Microbial Biotechnology* **26**: 1901-1912.

Aleksandrov, V.G., Blagodyr, R.N. and Ilev, I.P. 1967. Liberation of phosphoric acid from apatite by silicate bacteria, *Mikrobiolohichnyi Zhurnal (Kiev)* **29**: 111-114.

Cappuccino, J.C. and Sherman, N. 1992. Biochemical activities of microorganisms. In: *Microbiology*: *A Laboratory Manual* 3rd Edn. Benjamin/Cummings Publ., Co, Redwood City, California, USA, pp. 125-179.

Chakraborty, P., Balachandar, S. and Adrian, R.J. 2006. On the relationships between local vortex identification schemes. *Journal of Fluid Mechanics* **535:** 189-214.

Collins, C.H. and Lyne, P.M. 1980. *Microbiological Methods*, Butterwoth and Co. (Publishers) Ltd., London. pp. 17.

Goldstein, A.H. 1994. Solubilization of exogenous phosphates by gram negative bacteria. In: *Cellular and Molecular Biology of Phosphate and Phosphorylated Compounds in Microorganisms.* (Eds.) Silver *et al*, ASM Washington, D.C. pp. 197-203.

Han, H.S. and Lee, K.D. 2006. Effect of co-inoculation with Phosphate and Potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant, Soil Environment* **52**: 130-136.

Hu, X., Chen, J. and Guo, J. 2006. Two phosphate and potassium solubilizing bacteria isolated from Tianmu mountain, Zhejiang, China. *World Journal of Microbiology and Biotechnology* **22:** 83-990.

Jha, D. and Mathur, R.S. 1993. Combined effect of nitrogenous fertilizers and *Azospirillum brasilense* on the yield of nitrogen uptake by pearl millet. *International Journal of Tropical Agriculture* **11:** 31-35.

Lal, R. 2002. Soil carbon sequestration in China through agricultural intensification, and restoration of degraded and desertified ecosystems. *Land Degradation and Development* **13:** 469-478.

Loper, J.E. and Henckels, M.D. 1999. Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. *Applied and Environmental Microbiology* **65:** 5357-5363.

Mahadevan, A. and Sridhar, R. 1982. *Methods in Physiological Plant Pathology*. 4th edition, Sivakami publications, Chennai, India, 182 p.

Muralidharan, K., Reddy, C.S., Krishnadevi, D. and Laha, G.S. 2004. Field application of fluorescent pseudomonads products to control blast and sheath blight diseases in rice, *Journal of Mycology and Plant Pathology* **34**: 411-414.

Natarajan, T. and Buvana, R. 2000. Isolation and screening of phosphate solubilizing microorganisms. *Practical Manual- Microbial Interaction in Soil*, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, pp. 19-22.

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya* **17**: 362-370.

Ponmurugan, P. and Gopi, C. 2006. *In vitro* production of growth regulators and phosphate activity by phosphate solubilizing bacteria. *African Journal of Biotechnology* **5:** 348-350.

Ramarethinam, S. and Chandra, K. 2006. Studies on the effect of potash solubilizing bacteria *Frateuria aurantia* (Symbion -K- Liquid Formulation) on Brinjal (*Solanum melongena* L.) growth and yield, *Pestology* **11**: 35-39.

Sakthivel, N. and Gnanamanickam, S.S. 1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice, *Oryza sativa*, L. *Applied and Enrivonmental Microbiology* **53**(1): 2056-2059.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning*. Cold spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 49 -53.

Sheng, X.F. and Haung, W.Y. 2002. Mechanism of potassium release from feldspar affected by the strain of silicate bacterium. *Acta Pedologica Sinica* **39:** 863-871.

Sheng, X.F., Haung, W.Y and Yin, Y.X. 2003. Effects of application of silicate bacteria fertilizer and its potassium release. *Journal of Nanjing Agricultural University* **23:** 43-46.

Sheng, X.F. and He, L.Y. 2006. Solubilization of potassium-bearing minerals by a wildtype strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiol*ogy **52**: 66-72.

Sugumaran, P. and Janarthanam, B. 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Science* **3**(3): 350-355.

Tien, T.M., Gaskins, M.H. and Hubbell, D.H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37**(5): 1016-1024.

Vidhya, R.P., Nepolean, P., Balamurugan, A., Jayanthi, R., Beulah, T. and Premkumar, R. 2012. *In vitro* studies of biocontrol agents and fungicides tolerance against grey blight disease in tea. *Asian Pacific Journal of Tropical Biomedicine* S435-438.

Vijayabaskar, P., Babinastarlin, S., Shankar, T., Sivakumar, T. and Anandapandian, K.T.K. 2011. Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121). *Advances in Biological Research* **5**(2)**:** 71-76.

Wu, S.C., Cao, Z.H., Li, Z.G. and Cheung, K.C. 2005. Effect of biofertilizer containing N- fixer, P and K solubilization and AM fungi on maize growth: a green house trial. *Geoderma* **125**: 155-166.

Xiufang, H., Jishang, C. and Jiangfeng, G. 2006. Two Phosphate and Potassium solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World Journal of Microbiology and Biotechnology* **22**: 983-990.