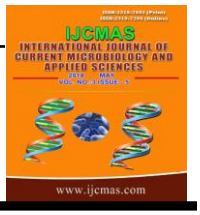
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 1045-1052**
http://www.ijcmas.com



Original Research Article

Studies on Potassium Solubilizing Bacteria from Southern Indian Tea Soils

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A B S T R A C T

Phosphate solubilizing bacteria (PSB) plays an important role in soil by solubilizing phosphorus and making it available to plants. PSB are potential solubilizers of bound phosphates in soil and vary in their ability to solubilize di and tricalcium phosphate. An attempt was made to isolate PSB from the rhizosphere soils of various field crops such as paddy, sorghum and groundnut. The isolates of PSB were screened based on the efficiency of solubilization of phosphorus and the production of growth promoting substances. Studies on the population density of PSB in tea soils indicated that the population was found to be 29.56 $x10^{-3}$ /gm soil dry wt in Coonoor soil samples and the same was 10.7 $x10^{-3}$ /gm soil dry wt in Chickmagalur soil samples. Total population was registered higher in Coonoor followed by Valparai and Munnar and lesser in Chickmagalur regions. Further it was correlated with soil nutrients like total organic carbon, total nitrogen and available phosphorous contents. Experiments were carried out to produce phytohormones such as Indole acetic acid (IAA) and Gibberellic acid (GA_3) using PSB under in vitro condition. The results indicated that all the strains of PSB were able to produce phytohormones such as IAA and GA_3 and among the two different plant growth regulators produced by KSB, IAA was found to be higher than GA_3.

K e y w o r d s

Phosphate solubilizing bacteria, Rhizosphere soils, Population density, Phytohormones

Introduction

Potassium (K) is a macronutrient in plants and also a major constituent of several soil minerals. It plays an important role in plant tolerance to various stress conditions such as cold, hot temperature, drought, pest and disease problems. Besides, it acts as catalyst for many of the enzymatic processes in plants that are necessary for plant growth. It has been reported that most of the K forms are insoluble in nature and has to be converted into soluble forms during plant growth by microbial consortia (Han and Lee, 2006). K is instrumental in plant metabolism by supplying energy required for metabolic processes (Laxmilal, 2002). Potassium deficiency occurs frequently in plants under stress periods in which yellowing of leaves, stunted growth, heavy flowering, scorching of leaf margins and die-back of the leaf tip could be noticed. Application of K enhances the pest and disease resistance in plants by strengthening of wood, leaf veins and guard cells in stomata and increasing the cuticle thickness in leaves (Chandra and Greep, 2006).

He *et al.,* (2006) studied the solubilization of K by *Bacillus edaphicus* and increased K uptake in wheat plants. Xiufang *et al.,* (2006)

isolated and characterized K solubilizing bacteria like *B. mucilagenosus* from mountain soils. Experiments were conducted to evaluate the potential of phosphorous solubilizing bacteria (PSB) *B. megaterium* and (KSB) *B. muciloginosus* in soils planted with eggplant (Han and Lee, 2006). Similarly, *Frateuria aurantia* belonging to the family Pseudomonaceae was obtained from the agricultural soils of Coimbatore region of Tamil Nadu (Ramarethinam and Chandra, 2006). On the other hand, co-inoculation of PSB and KSB were increased the mineral uptake and enhanced the growth of pepper and cucumber very significantly (Han and Lee, 2006). Further they were reported that both PSB and KSB possess the ability to solubilize insoluble inorganic phosphorous and potassium; respectively and make it available to plants. This solubilization effect is generally due to the production of organic acids and enzymes by these organisms. They are also known to produce amino acids, vitamins and growth promoting substances like IAA and GA_3 which help in better growth of the plants (Ponmurugan and Gopi, 2006a).

Numerous commercial formulations of biofertilizer produced by various Agro companies are available in the market. These formulations may not survive in tea fields because formulations containing bioinoculants might have obtained from different crop soils. By virtue of tea plants being cultivated in acidic soils, indigenous strains of KSB would respond to the productivity in tea plantations significantly. In this context, studies were conducted to enumerate the population density of KSB in tea rhizosphere soils and correlated with soil nutrient factors. Efforts were also made to isolate and identify KSB and subsequently the production of growth regulators and K solubilizing potential by KSB were also attempted under *in vitro* condition.

Materials and Methods

Collection of soil samples

Soil samples were collected from different tea planting districts of southern India such as Valparai (Anamallais), Coonoor (Nilgiris), Vandiperiyar (Central Travancore), Meppadi (Wynaad), Munnar (High Range) and Chickmagalur (Karnataka) for isolation of KSB. The rhizosphere soil samples were obtained from different fields planted with UPASI-3 clone after removing soil debris. These samples were allowed to air dry at room temperature and various parameters like soil pH, total organic carbon (Walkley and Black, 1934), total nitrogen (AOAC, 1990), available phosphorous (Jackson, 1973) and exchangeable potassium (Peach and Tracey, 1956) were determined subsequently.

Isolation and characterization of KSB

Enumeration and isolation of KSB present in these soil samples were performed by serial dilution plate technique using Aleksandrov medium (Aleksandrov *et al.*, 1967). Bacterial colonies causing clear K solubilizing halo-zones by a turbid background were selected and purified for further study (Fig. 1A and B). Inoculated cultures were incubated at 37°C in an incubator shaker with gentle agitation (200 rpm) to maintain aerobic condition for 3-5 days depending on the type of experiment. Biochemical characterization such as pigment production, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction, indole production, gelatin hydrolysis, and hydrogen sulphide production were carried out to identify the genus of KSB. Gram's staining was carried out using crystal violet solution followed by flagella stain (James and Sherman, 2004).

Effect of biotic and abiotic factors on growth of KSB

Influence of abiotic factors such as pH (4.0 to 6.5) and temperature (5 to 40°C) and biotic factors such as carbon and nitrogen sources on growth of KSB were studied. Seven different carbon compounds such as glucose, fructose, maltose, sucrose, starch and cellulose and four nitrogen compounds such as ammonium nitrate, sodium nitrate, potassium nitrate and casein hydrolysate were added by replacing starch and potassium nitrate respectively in the basal medium.

NaCl tolerance was determined by supplementing with various NaCl concentrations in Aleksandrov medium. The inoculated plates were incubated for 5-10 days depending upon the nature of experiment.

Estimation of Plant growth regulators

Experiments were conducted to know whether the KSB have the capacity to produce plant growth regulators such as Indole acetic acid (IAA) and Gibberelic acid (GA_3). Five days old cultures of KSB were transferred to Aleksandrov broth containing L-Tryptophan as a substrate for the production of IAA and GA_3. After ten days of incubation, culture filtrates were taken for the estimation of IAA and GA_3 contents according to the procedure described by Tien *et al.,* (1979) and Mahadevan and Sridhar (1996) respectively.

Determination of Potassium solubilizing efficiency of KSB

Hundred mL of Aleksandrov broth amended with different K sources such as Potassium Aluminum Silicate (PAS), Murate of Potash (MOP) and Sulphate of Potash (SOP) was taken in 250 mL Erlenmeyer flask and inoculated with KSB. It was incubated at 37°C for 10 days in an incubator shaker (200 rpm).

After incubation, culture filtrates were centrifuged at 8000 g for 10 min. The supernatants were subjected to estimate the release of potassium by KSB using a Flame photometer (Peach and Tracey, 1956). Autoclaved medium without inoculation of KSB served as control.

Results and Discussion

Population density

Total number of KSB population present in tea soils indicated that the population was found to be 29.56 $x10^{-3}$ /gm soil dry wt in Coonoor and 10.7 $x10^{-3}$ /gm soil dry wt in Chickmagalur region. Total population was found to be more in Coonoor region followed by Valparai and Munnar and lesser in Chickmagalur regions. The population density was significantly coincided with soil nutrients like total organic carbon, total nitrogen and available phosphorous contents (Table 1). Similar observations were reported in tea soils by Baby *et al.,* (2002) who observed a correlation between beneficial microorganisms and nutrients in the soil. Ponmurugan *et al.,* (2007) studied actinomycetes diversity in southern Indian tea soils recently. It has been reported that most of the beneficial microorganisms tend to grow in acidic soils which is an important characteristic features and with adequate source of carbon and nitrogen present in it that enhance the rate of degradation (Stackebrandt *et al.,* 1991).

Characterization of KSB

A total of 36 strains of KSB (6 strains from each agroclimatic zone) were obtained from soil samples and subjected to screen for their potential of K solubilizing activity. Out of these, one strain from each agroclimatic zone was selected for further studies. The isolates were designated as VKSB, CKSB, VKSB1, MKSB, MKSB1 and CKSB1 (Table 1).

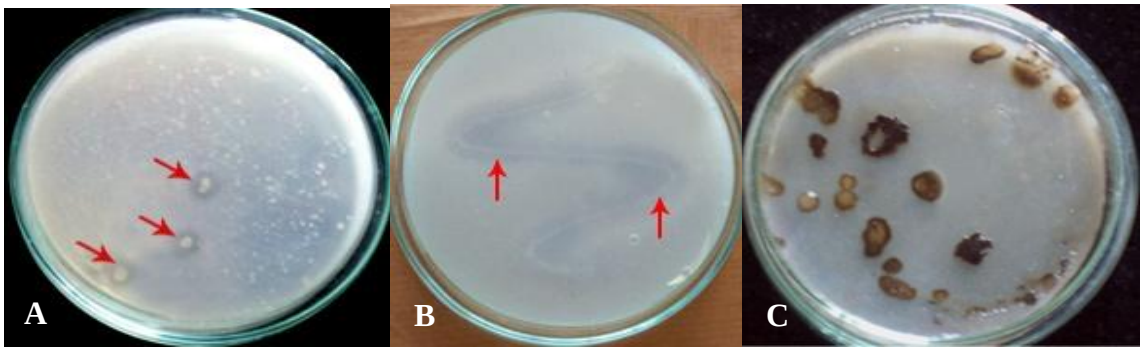
Morphological and biochemical characteristics of the strains were studied and results were presented in Table 2. The results indicated that the purified strains of KSB belonged to *Bacillus* sp. as they showed positive response to Gram's staining, catalase, amylase, hydrogen sulphide production, methyl red and carbohydrate fermentation and negative to casein hydrolysis, oxidase, indole production, gelatin and voges proskauer tests. All the strains had typical large capsules with endospores but no flagella were observed. They were found to be rod shaped gram-positive organism and they were smooth, round, convex, slimy, elastic, translucent and non-motile in nature (Holt, 1989). These results were coincided with the report of Han and lee (2006). VKSB and CKSB strains were found to be brown pigmented colonies (Table 2 and Fig. 1C).

Table.1 Population density of KSB and nutrient status in southern Indian tea soils

Name of Agroclimatic zones	Designation of strains	Population Density *	Soil pH	Organic carbon (%)	Total Nitrogen (%)	Available P (ppm)	Available K (ppm)
Valparai	VKSB	22.5	4.7	3.8	2885	16.0	55.5
Coonoor	CKSB	29.5	5.0	3.8	3830	16.6	95.0
Vandiperiyar	VKSB1	14.7	4.8	2.9	2937	11.8	42.7
Munnar	MKSB	22.5	5.1	3.3	3030	11.0	62.4
Meppadi	MKSB1	12.3	4.6	3.4	3400	15.5	50.0
Chickmagalur	CKSB1	10.7	5.0	2.9	2920	13.8	43.8
SE ±		5.03	0.67	0.82	18.59	5.23	8.25
CD at P=0.05		10.25	1.38	1.02	23.68	5.44	12.7

* cfu x 10^{-3} /gm soil dry wt

Fig.1 Growth of KSB in Aleksandrov agar medium



[A: KSB colonies (arrows indicate K solubilizing halo-zone around bacterial colonies); B: Growth of CKSB strain by streaking method (arrows indicate K solubilizing halo-zone); C: Brown pigmented colonies]

Table.2 Characterization of KSB strains

S.No	Parameters	Isolates of Potassium solubilizing bacteria					
		VKSB	CKSB	VKSB1	MKSB	MKSB1	CKSB1
1.	Gram's staining	+	+	+	+	+	+
2.	Capsule staining	+	+	+	+	+	+
3.	Cell shape	rod	rod	rod	rod	rod	rod
4.	Motility test	-	-	-	-	-	-
5.	Pigment production	+	+	-	-	-	-
6.	Starch hydrolysis	+	-	-	+	+	+
7.	Casein hydrolysis	+	-	-	+'	+'	-
8.	Catalase	+'	+	+	+'	+	+
9.	Amylase	+	+	+	+	+	+
10.	Oxidase	-	-	-	+'	-	-
11.	Nitrate reduction	+	+	+	+	-	-
12.	Indole production	-	-	-	-	-	-
13.	Gelatin hydrolysis	-	-	-	-	+'	-
14.	Hydrogen sulphide production	+	+	+	+	+'	+'
15.	Methyl red	+	+	+	+	+	+
16.	Voges Proskauer	-	-	-	-	+'	-
17.	Carbohydrate fermentation	+	+	+	+	+	+

+ Positive; +' Weekly positive; - Negative

Table.3 Effect of biotic and abiotic factors on the growth of KSB strains

Parameters	Isolates of Potassium solubilizing bacteria					
	VKSB	CKSB	VKSB1	MKSB	MKSB1	CKSB1
Optimum pH	5.0	5.0	5.5	5.5	5.0	6.0
Optimum temperature (°C)	30	30	25	25	30	30
Optimum NaCl concentration (%)	2.5	2.5	3.0	3.5	3.5	2.0
Glucose	-	+'	+'	-	-	+
Fructose	+	+'	+'	+	+	+
Maltose	+	+	+	-	+'	+'
Sucrose	+	+	-	-	+	+
Starch	+	+	+	+	+	+
Cellulose	+	+	-	-	+	-
Ammonium nitrate	-	+	-	-	+'	+'
Sodium nitrate	+	+'	+'	+'	+	+'
Potassium nitrate	+	+	+	+	+	+
Casein hydrolysate	+	+'	+'	+'	+	+

+ Positive; +' Weekly positive; - Negative

Table.4 *In vitro* production of plant growth promoting substances and K solubilizing potential of KSB

Name of the strain	Growth promoting substances (ppm)*		K solubilizing potential in different K sources (mg/ml)*		
	IAA	GA_3	PAS	MOP	SOP
VKSB	30.07	11.48	1.88	2.52	1.89
CKSB	36.31	14.88	3.25	4.87	3.01
VKSB1	30.51	12.16	1.90	2.50	1.81
MKSB	33.90	14.00	2.95	3.71	3.04
MKSB1	32.15	13.11	2.41	3.47	2.41
CKSB1	30.08	10.73	1.78	2.00	1.65
SE ±	1.05	1.22	0.88	0.89	0.80
CD at P=0.05	2.89	3.04	1.08	1.22	1.14

IAA: Indole acetic acid; GA_3: Gibberelic acid
PAS: Potassium Aluminium Silicate; MOP: Murate of Potash; SOP: Sulphate of Potash
* On 10^{th} day

The growth of KSB strains upon Aleksandrov medium adjusted with different pH revealed that a better growth was recorded between pH 5.0 and 5.5. The optimum pH was 5.5 for all the strains except CKSB1 strain which showed 6.0 (Table 3). This pH level may be correlated with the soil pH. The optimum temperature for the growth of KSB strains was 30°C followed by 25°C. The optimum temperature was 30°C for majority of the strains except VKSB1 and MKSB strains where it was found to be 25°C. Among the different carbon sources tested, starch was found to be suitable for maximum growth followed by maltose and fructose. On the other hand, potassium nitrate was found to be suitable for optimum growth followed by sodium nitrate and casein hydrolysate (Table 3). Solubilization and mobilization of K by KSB have been known to be influenced by the components of medium and cultural conditions such as pH, temperature, carbon and nitrogen sources (Augustine *et al.,* 2004).

All the strains of KSB were able to produce phytohormones such as Indole acetic acid (IAA) and Gibberellic acid (GA_3). Among the two different plant growth regulators produced by KSB, IAA was found to be higher than GA_3. Similarly, among the six strains tested for the production of PGPRs, CKSB strain was better than the other strains of KSB. CKSB strain produced 36.31 ppm of IAA and 14.88 ppm of GA_3 on 10^{th} day of incubation. The bacterial cultures release greater quantities of IAA and GA_3 in the presence of a physiological precursor, tryptophan in a culture medium. Production of IAA and GA_3 varies greatly among different species and is also influenced by culture conditions, growth stage and availability of substrate(s) (Vijila, 2000). Tien *et al.,* (1995) reported that *Azospirillum* and Phosphobacteria isolated from the soil of pearl millet produced IAA, GA3 and cytokinin like substances which ultimately enhanced the plant metabolism. PSB isolated from rhizosphere soils of different food and forage crops are known to produce growth regulating substances and some of them are capable of dissolving inorganic phosphate (Ponmurugan and Gopi, 2006b).

All the strains of KSB were able to solubilize inorganic potassium to available form. However, there was a positive correlation between K solubilization and growth regulator biosynthesis (Table 4). There is an increasing evidence that KSB could improve plant growth due to biosynthxesis of plant

growth substances rather than their action upon the release of available K. All the strains of KSB could solubilize K sources such as PAS, MOP and SOP very effectively under *in vitro* condition. Among the three different forms of K tested for K solubilization, MOP was solubilized more followed by SOP and PAS. CKSB strain was solubilized more K in the medium followed by MKSB and VKSB, VKSB1 and CKSB1 strains were found to be the least in terms of K solubilization. Sheng and Haung (2002) reported that bioinoculants showed negative result for Voges-Proskauer reaction, positive for methyl red test, indicating the production of some organic acids which helps K solubilization. It is generally observed that the principal mechanism of K solubilization is the action of organic acids synthesized by KSB (Welch and Vandevivere, 1994).

From the study it may be concluded that an easy, reproducible and reliable culturing technique was standardized for the isolation and identification of KSB from tea soils. Further the present study could be useful for preparing carrier based bioformulations containing indigenous KSB to enhance the uptake of K by the plants and thereby increase the yield potential in tea plantations.

References

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.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. (ed), Helrich, K. 15th Edition, Vol 1&2. AOAC Inc. USA.

Augustine, S.K., Bhavsar, S.P., Baserisalehi, M. and Kapadnis, B.P. 2004. Isolation, characterization and optimization of antifungal activity of an actinomycete of soil origin. *Indian J. Expri. Biol*. 42: 928-932.

Baby, U.I., Tensingh Baliah, N., Ponmurugan, P. and Premkumar, R. 2002. Population level of certain of certain beneficial microorganisms in tea soils. *UPASI Tea Res. Found. Newsletter* 12: 3.

Chandra, K. and Greep, S. 2006. *Potassium mobilizing bacteria* (*Frateuria aurentia*). Regional centre of organic farming, Bangalore, Karnataka, 72p.

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 Environ*.52: 130-136.

He, L.Y., Sheng, X. and Xia, F.A.N.G. 2006. Solubilization of potassium- bearing minerals by a wild- type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Can. J. Microbiol*. 52: 66-72.

Holt, J.G. 1989. *Bergey's manual of systemic bacteriology*, Vol. 4. (eds) S.T. Williams and M.E. Sharpe, Baltimore, Cambridge University Press. UK.

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall of India Pvt. Ltd. New Delhi, pp. 498-516.

James, G. P. and Sherman, N. 2004. *Microbiology Laboratory Manual*. 6th edition, Pearson Education, Singapore, 491p.

Laxmilal, L. 2002. *Phosphatic Biofertilizers*. Agrotech, Publ. Academy, Udaipur, India, 224p.

Mahadevan, A. and Sridhar, R. 1996. *Methods in Physiological Plant Pathology*. 4th Edition, Sivakami Publications, Chennai, Tamil Nadu. 182p.

Peach, K. and Tracey, M.V. 1956. *Modern Methods in Plant Analysis*. Vol. IV. Springer-Verlag, Germany.

Ponmurugan, P. and Gopi, C. 2006a. *In vitro*

production of growth regulators and phosphate activity by phosphate solubilizing bacteria. *African J. Biotechnol*. 5: 348-350.

Ponmurugan, P. and Gopi, C. 2006b. Distribution pattern and screening of Phosphate solubilizing bacteria isolated from different food and forage crops. *J. Agron.* 5: 600-604.

Ponmurugan, P., Gopi, C. and Maripandi, A. 2007. Studies on Actinomycetes diversity in southern Indian tea soils for antifungal activity. *J. Plantn. Crops* 35: 28-32.

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.

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.

Stackebrandt, E., Witt, D., Kemmerling, C., Kroppenstedt, M. and Liesack, W. 1991. Designation of *Streptomycete* 16S and 23S rRNA based target regions of oligonucleotide probes. *Appl. Environ. Microbiol*. 57: 1468-1477.

Tien, M., Kersten, P.J. and Kirk, T.K. 1987. Selection and improvement of lignin degrading microorganisms: potential strategy based on lignin model-amino acid adducts. *Appl. Environ. Microbiol*. 53: 242-245.

Vijila, K. 2000. Estimation of IAA production in nitrogen fixing microorganisms. In: *Practical Manual in Microbial Interaction in Soil*. Tamil Nadu Agricultural University, Coimbatore. pp 38-39.

Walkley, A. and Black, C.A. 1934. An examination of the Degtjareff method for determining soil organic matter and proposed modification of chromic valid titration method. *Soil Sci*. 37: 29-38.

Weklch, S.A. and Vandevivere, P. 1994. Effect of microbial and other naturally occurring polymers on minerals dissolution. *Geomicrobiology J.* 12: 227-238.

Xiufang H., Jishuang C. and Jiangfeng G. 2006. Two Phosphate and Potassium solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J. Microbiol. Biotechnol*. 22: 983-990.