J. Sci. Trans. Environ. Technov. 2020, 13(3) : 106-109

Screening of Potassium Solubilizing bacteria and their growth promoters

Gelli Veena Shravanthi[1], Prabakaran Panchatcharam[1], A.S. Shijila Rani[2] and V. Ambikapathy[2]

https://doi.org/10.56343/STET.116.013.003.003
http://stetjournals.com

Article History
Received: 13.08.2018
Revised and Accepted : 11.12.2019
Published: 16.03.2020

Abstract

Potassium is a vital component of plant nutrition package limiting crop yield and quality that performs a multitude of important biological functions to maintain plant growth. The isolation of potassium solubilizing bacteria (PSB) were carried out using soil sample of *Vicina faba* plant rhizospore samples. The bacterial isolates were selected exhibiting highest potassium solubilization and were characterized on the basis of colony morphology and biochemical characters. The screening of PSB was on the basis of growth diameters and zone formation. Among the six PSB screened, the PSB6 was having maximum activity as well as more zone formation. The plant hormones are a group of naturally occurring, organic substances which influence the physiological processes. PSB_1 to PSB6 strains have maximum potential for production of plant growth hormone such as Indole acetic acid and Gibberellic acid. The Indole acetic acid synthesized from PSB_1 bacterial strains stimulates primarily in leaf primordia and young leaves and in developing seeds. It was 102.5, 98.9, 72.7, 33.4, 121.2, 176.4 and 179.4µg/ml for PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 strains, respectively. The Gibberlic acid production was 108, 78, 89, 56, 121 and 159µg/ml for PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 bacterial strains, respectively. The siderophores are molecular receptors that binds and transports iron content. The siderophore level was 1.6, 3.9, 8.9, 1.4, 0.8 and 0.4µg/ml for the potassium solubilizing strains PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 respectively. Among all the strains, PSB_3 was excellent in the iron binding activity when compared to other strains whereas hydrogen cyanide (HCN) was tested positive with the strains PSB_1, PSB_2, PSB_3 and PSB_4.

Key words: HCN, Potassium, PSB, *Rhizobium*, Siderosphore.

Gelli Veena Shravanthi

email: *veenagupta89@gmail.com*

[1]PG and Research Department of Microbiology, Marudupandiyar College, Vallam, Thanjavur, Tamil Nadu, India.

[2]PG and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India

INTRODUCTION

Potassium (K) is seventh most common element in the earth's crust. It constitutes about 2.5 per cent of the lithosphere. However, actual soil concentrations of this nutrient vary widely ranging from 0.04-3.00 per cent (Sparks and Huang, 1985; Shang and Huang, 2002). Highest proportions of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994) such as micas, illite, feldspar and orthoclase. Potassium plays a foremost role in translocation of carbohydrates, photosynthesis, water relations, resistance against insects and diseases and sustains balance between monovalent and divalent captions (Brar and Tiwari, 2004). Deficiency of K is not as wide spread as that of nitrogen and phosphorus but with the introduction of high yielding varieties and hybrids during green revolution and with the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate. Extensive use of chemical fertilizers is proven to destroy soil structures as well as aggravate environmental pollution by contaminating underground water. Many types of microorganisms are known to inhabit soil, especially rhizosphere and play an important role in plant growth and development. K-solubilizing bacteria that are able to release potassium from insoluble minerals.

MATERIALS AND METHODS

Collection of soil samples

Samples of soil and root system from healthy rhizosphere of *Vicina faba* were collected in sterile plastic bags from ten plants each at random from the field. Each sample consisted of 100 g soil and *Rhizobium* sp. from the soil sample was isolated.

Screening of potassium solubilization by *Rhizobium* sp.

Potassium solubilization by rhizobacterial isolates was studied on modified Aleksandrov medium plates by the spot test method. Plates of modified Aleksandrov medium (A) having mica powder (insoluble form of potassium) and medium (B) having soluble form of potassium i.e., K_2HPO_4 were prepared. A loopful of 48-hour old growth of the rhizobacterial strain (10 ìL of

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

10^6 CFU mL^{-1}) was spotted on above prepared plates. Ten bacterial cultures were spotted on each plate and cultures were spotted in same sequence on both types of media plates. Plates were incubated at 28±2 ^{0}C for 3 days. Detection of potassium solubilization by different rhizobacterial isolates was based upon the ability of solubilization and zone formation.

Estimation of Indole Acetic Acid (IAA) production

For the detection of Indole acetic acid production by Sarwer and Kremer (1995) method was used. One ml of supernatant was taken, equal amount of Salkowsky's reagent was added, and incubated for 30 minutes and OD was taken at 536 nm. Amount of IAA produced was estimared from the standard graph .

Estimation of Gibberllic acid production

Production of gibberellic acid was detected by spectrophotometric method. Forty eight hours old growth of bacterial culture was centrifuged at 10,000 rpm for 15 - 20 min. The pH value of supernatant was adjusted to 2.5 using stock 3.75 N HCl. Supernatant was extracted using liquid-liquid (ethyl acetate/ $NaHCO_3$) extraction method. The amount of gibberellic acid in the ethyl acetate phase was measured by the UV spectrophotometer at 254 nm (Berryos *et al*., 2004).

Estimation of Siderophore production

Chrome azurols (CAS) assay solution was used for the detection of siderophore following Schwyn and Neilands (1978). 0.5 ml supernatant was collected from each sample and 0.5 mL H_2SO_4 was added and allowed to cool. One ml of 1 % sulphanilic acid and 0.5 ml of 1.3 % Iodine solution were added and allowed to stand for 5 min. The excess of iodine was destroyed by adding 1 ml of 2 % sodium arsenate solution and allowed to stand till yellow colour disappears. One ml of 0.3 % á-napthylamine solution was added and incubated for 30 min till pink colour develops. The OD was taken at 536 nm. Amount of siderophore produced was estimated from the standard graph.

HCN production

Picrate assay (Castric, 1974) was followed for the qualitative analysis of hydrocyanic acid production. Nutrient agar slants were sreaked with isolates. After streaking a filter paper strip impregnated with 0.5% picric acid and 2.0% sodium carbonate was suspended above the medium. The slants were incubated for 24 hrs at 37^{0}C with picric acid indicator papers placed inside the lids. After incubation, the change of filter paper from yellow to orange brown indicated the presence of cyanide.

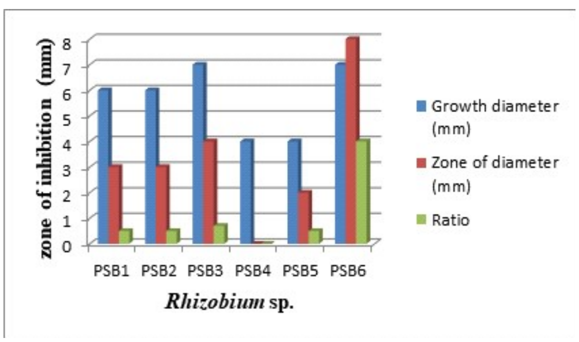

Fig. 1. Screening of Potassium solubilizing for *Rhizobium* sp. by plate assay

Table 1. Screening of Potassium solubilizing for *Rhizobium* sp. by plate assay

S. No.	Isolates	Growth diameter (mm)	Zone diameter (mm)	Ratio
1	PSB_1	6	3	0.5
2	PSB_2	6	3	0.5
3	PSB_3	7	4	0.7
4	PSB_4	4	-	-
5	PSB_5	4	2	0.5
6	PSB_6	7	8	4

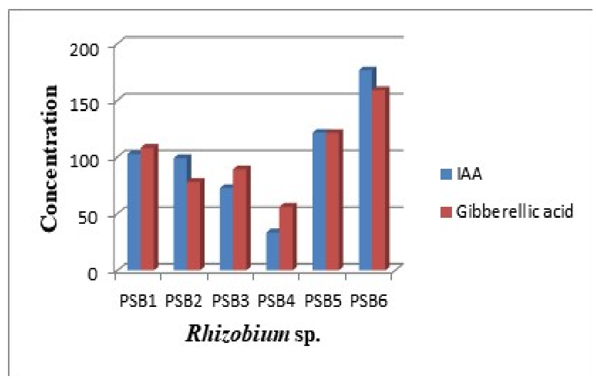

Fig. 2. Estimation of plant growth hormone from *Rhizobium* sp.

Table 2. Estimation of plant growth hormone by *Rhizobium* sp.

S.No.	Isolates	PGPR (µg/mL^{-1})	
		IAA	Gibberellic acid
1	PSB_1	102.5	108
2	PSB_2	98.9	78
3	PSB_3	72.7	89
4	PSB_4	33.4	56
5	PSB_5	121.2	121
6	PSB_6	176.4	159

RESULTS AND DISCUSSION

Six strains of potassium solubilizing bacteria (PSB), PSB_1 to PSB_6 strains of *Rhizobium* with growth diameters of 6, 6, 7, 4, 4 and 7 mm and with zone diameter of 3, 3, 4, 0.2 and 8 mm with the ratio of 0.5, 0.5, 0.7, 0.5 and 4.0% were screened from the assay of rhizosphere of *Vinica faba* (Table 1 and Fig.1).

Among the K bearing silicate minerals mica was found to be solubilized readily than other minerals (Sugumaran and Janarthanam, 2007; Mikhailouskaya and Tehernysh, 2005). Similar results were also reported by Archana *et al.* (2008) on KSB, who found that many species of *Bacillus* and *Pseudomonas* were able to solubilize mica and gave zone of solubilization in solid media. Some potassium solubilizing rhizobia (KSR) *Agrobacterium tumefaciens* OPVS11 (*Zea mays*) and *Rhizobium pusense* OPVS6 (*Saccharum officinarum*) were found to dissolve waste mica (Meena *et. al.*, 2015). The results obtained from the present study are in agreement with that of other investigators who reported that *Bacillus megaterium* (Hu and Boyer, 1996) and *B. mucilaginosus* (Biswas and Basak, 2014) were capable of solubilizing mica in appreciable amounts. Archana *et al.* (2008) reported that KSB *Bacillus sp*. to solubilize 44.49 ìg/ml mica in liquid medium.

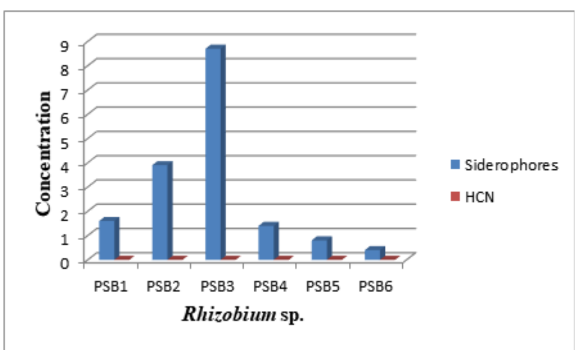

Fig. 3. Analysis of Siderophore production and HCN production from *Rhizobium* sp.

Table 3. Analysis of Siderophore production and HCN production from *Rhizobium* sp.

S.No.	Isolates	PGPR (µg/mL^{-1})	
		Siderophores	HCN
1	PSB_1	1.6	+
2	PSB_2	3.9	+
3	PSB_3	8.7	++
4	PSB_4	1.4	+
5	PSB_5	0.8	-
6	PSB_6	0.4	-

(+) growth (-) no growth

In the current research, 102.5, 98.9, 72.7, 33.4, 121.2 and 176.4 mg/ml of acetic acid production were recorded from the strains, PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6, respectively., whereas Gibberellic acid production from the strains PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 were108, 78, 89, 56, 121 and 159 µg/ml, respectively (Table 2 and Fig.2).

With the introduction of high yielding crop varieties/ hybrids and the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate. Moreover, due to imbalanced fertilizer application, potassium deficiency is becoming one of the major constraints in crop production. This emphasized the search to find an alternative indigenous source of K for plant uptake and to maintain K status in soils for sustaining crop production (Supanjani *et al.*, 2006, Sindhu *et al.*, 2012). Soil microbes have been reported to play a key role in the natural K cycle and therefore, potassium solubilizing microorganisms present in the soil could provide an alternative technology to make potassium available for uptake by plants (Groudev, 1987, Rogers *et al.*, 1998) Thus, identification of microbial strains capable of solubilizing potassium minerals quickly can conserve our existing resources and avoid environmental pollution hazards caused by heavy application of chemical fertilizers.

In the current research, the production of siderophores were 1.6, 3.9, 8.7, 1.4, 0.8 and 0.4 µg/ml by PSB_1, PSB_2, PSB_3, PSB_4,PSB_5 and PSB_6 strains of *Rhizobium*,respectively. On the other hand, the PSB_1, PSB_2, PSB_3, and PSB_4, strains of *Rhizobium* sp. HCN,which plays an essential role in physiological functions. From this study, *Vinica faba* plant rhizosphere, six plant growth promoting strains of Rhizobacteria (PGPR) were isolated from the soil which could be directly attributed to the beneficial effects of biological nitrogen fixation and phytohormone production and indirectly to phosphate and potassium solubilization, iron siderosphores and hydrolyzing enzyme production for the growth of *Vicina faba* plant (Table 3 and Fig. 3).

Currently, little information is available on potassium solubilization by bacteria, their mechanisms of solubilization and effect of KSB inoculation on nutrient availability in soils and growth of different crops (Sheng and Huang, 2002). Potassium release from the minerals might be affected by pH, oxygen and the bacterial strains used. The efficiency of potassium solubilization by different bacteria might vary with the nature of potassium bearing minerals and aerobic conditions, as well. The extent of potassium solubilization by *B. edaphicus* in the liquid media was more and better growth was observed on illite than feldspar. Therefore, there are immense possibilities for further increasing the production of crops by application of K-bearing rock materials and potassium solubilizing bacteria as biofertilizers.

ACKNOWLEDGEMENT

The authors are very much thankful to the principal, Marudupandiyar College, Thanjavur and Indian Biotrack Research Institute, Thanjavur (Dist), Tamil Nadu for providing lab facilities to do this work.

REFERENCES

Aneja, K.R. 2002. Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom production technology, New Age International (P) Ltd., New Delhi.

Annonymous. 1957. Manual of Microbiological Methods, McGraw Hill Book Co., *Inc., New York*, pp.127.

Archana, D.S.; Savalgi, V.P. and Alagawadi, A.R., (2008). Effect of potassium solubilizing bacteria on growth and yield of maize. *Soil Biol. Ecol.*, **28**(1-2): 9-18.

Biswas, D.R., and Basak, B.B., (2014). Mobilization of potassium from waste mica by potassium solubilizing bacteria (*Bacillus mucilaginosus*) as influenced by temperature and incubation period under *invitro* laboratory conditions. *Agrochimica*. **4**: 309-320.
https://doi.org/10.12871/0021857201442

Brar, M.S., and Tiwari, K.S., (2004). Boosting seed cotton yield in Punjab with potassium. Better Crops, *J. Plant Nutr. Soil Sci.*, **168**: 521-530.

Goldstein, A.H., (1994). Involvement of the quino protein glucose dehydrogenase in the solubilization of exogeneous mineral phosphates by gram negative bacteria. In phosphate in micro-organisms: cellular and molecular biology. *Cell. Mol. Biol.*, Eds., pp.197-203.

Groudev, S.N., (1987). Use of heterotrophic microorganisms in mineral biotechnology. *Acta Biotechnol.*, 7, 299-306.
https://doi.org/10.1002/abio.370070404

Hu, X., and Boyer, G.L., (1996). Siderophore mediated aluminium uptake by *Bacillus megatherium* ATCC 19213. *Appl. Environ. Microbiol.*, **62**: 4044-4048.
https://doi.org/10.1128/aem.62.11.4044-4048.1996
PMid:16535439 PMCid:PMC1388977

Hu, X.F., Chen, J., and Guo, J.F., (2006). Two phosphate and potassium solubilizing bacteria isolated from Tiannu mountain, Zhejiang, China. *World J. Microbiol. Biotechnol.*, 22, 983-990.
https://doi.org/10.1007/s11274-006-9144-2

Meena, V.S.; Maurya, B.R.; Verma, J.P.; Aeron, A.; Kumar, A.; Kim, K., and Bajpai, V.K., (2015). Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and Krelease dynamics from waste mica. *Ecol. Engineering*, **81**: 340–347.
https://doi.org/10.1016/j.ecoleng.2015.04.065

Mikhailouskaya, N., and Tcherhysh, A., (2005). K-mobilizing bacteria and their effect on wheat yield. *Latvian J. Agron.*, **8**: 154-157.

Rogers, J.R., Bennett, P.C., and Choi, W.J., (1998). Feldspars as a source of nutrients for microorganisms. *American Minerology*, 83, 1532-1540.
https://doi.org/10.2138/am-1998-11-1241

Sarwer, M., and Kremer, R.J., (1995) "Enhanced suppressin of plant growth through production of L-tryptophan derived compounds by deleterious rhizobacteria" *Plant and soil*; 261-269.
https://doi.org/10.1007/BF00011328

Schwyn, B., and Neilands, J.B., (1978). "Universal Chemical assay for detection and determination of siderophores" *Anal. Biochem*- 160, pp. 40-47.
https://doi.org/10.1016/0003-2697(87)90612-9

Sparks and Huang, P.M., (1985). Physical chemistry of soil potassium. In Potassium in agriculture (ed.) Munson, R.D., *American Soc. Agron. J.*, pp.201-276.
https://doi.org/10.2134/1985.potassium.c9

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

Sheng, X.F., and Huang, W.Y., (2002). Study on the conditions of potassium release by strain NBT of silicate bacteria. *Scientia Agricultura Sinica*, 35, 673-677.

Supanjani, Han, H.S., Jung, S.J. and Lee, K.D., (2006). Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. *Agronomy and Sustainable Development*, 26, 233-240.
https://doi.org/10.1051/agro:2006020

Sindhu, S.S., Parmar P., and Phour, M., (2012). Nutrient cycling: potassium solubilization by microorganisms and improvement of crop growth. *In:* Geomicrobiology and biogeochemistry: Soil biology. Parmar, N. and Singh, A., Eds. *Springer-Wien/New York, Germany*; (2);1-8.