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Screening of potassium solubilizing bacteria and their growth promoters

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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 solubility bacteria (PSB) were carried out using soil sample of *Vicina faba* plant rhizospore samples. The bacterial isolates were selected exhibiting highest potassium solubilization and characterization on the basis of colony morphology and biochemical characters. The screening of PSB on the basis of growth diameters and zone formation, were measured. Among the six PSB, the PSB6 was maximum activity as well as zone formation than followed by other strains. The plant hormones are a group of naturally occurring, organic substance which influencing the physiological processes. PSB_1 to PSB6 strain has maximum potential for production of plant growth hormone such as Indole acetic acid and Gibberellic acid was determined. The indole acetic acid synthesized from PSB_1 bacterial strains which stimulatory primary in leaf primordial 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 with PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 strains reported respectively. Whereas Gibberlic acid was 108, 78, 89, 56, 121 and 159µg/ml with PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 bacteria were estimated respectively. The siderophores are molecular receptor that binds and transports iron content. It was 1.6, 3.9, 8.9, 1.4, 0.8 and 0.4µg/ml and the potassium solubilizing strain of PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 performed respectively. Among the all the strains, PSB_3 was excellent production of iron binding activity when compared with other strains whereas hydrogen cyanide (HCN) is a electroplating, chemical synthesis and production of synthetic fibers between potassium and PSB_1, PSB_2, PSB_3 and PSB_4 was tested and performed the structural functioning when compared with other strains of rhizobium bacteria.

Keywords: potassium, PSB, siderophore, *Rhizobium*. HCN

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) [15]. Highest proportions of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994) [6] 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) [5]. 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 are able to release potassium from insoluble minerals.

Materials and Method
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 isolation of *Rhizobium* sp. from the soil sample.

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 µL of 106 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 medium plates. Plates were incubated at 28±2 for 3 days. Detection of potassium solubilization by different rhizobacterial isolates on the based upon the ability of solubilization of zone formation.

Estimation of IAA production

For the detection of Indole acetic acid production by Sarwer and Kremer (1995) method was used. 1 mL of supernatant and add equal amount of Salkowsky's reagent was added. Incubate for 30 min and take OD at 536 nm. Amount of IAA produced from the standard graph was estimated.

Estimation of Gibberllic acid production

Production of gibberellic acid was detected by spectrophotometric method. 48 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.

Estimation of Siderophore production

Chrome azurols (CAS) assay solution was used for the detection of siderophore was followed by Schwyn and Neilands (1978) [14]. 0.5 ml supernatant was collected from each sample and 0.5 mL H_2SO_4 was added. Allow to cool. Add 1 mL of 1 % sulphanilic acid and 0.5 ml of 1.3 % Iodine solution. Allow to stand for 5 min. Destroy excess of iodine by adding 1 mL of 2 % sodium arsenate solution. Wait till yellow colour disappears. Add 1 mL of 0.3 % α-napthylamine solution and incubate for 30 min till pink colour develops. Take OD at 536 nm. Amount of siderophore produced from the standard graph was estimated.

HCN production

Picrate assay was followed for the qualitative analysis of hydrocyanic acid production. Streak nutrient agar slants with isolates. After streaking a filter paper strip impregnated with 0.5% picric acid and 2.0% sodium carbonate suspended above the medium. Incubate the slants for 24 hrs at 37˚C. Incubating heavily inoculated nutrient agar plates in an inverted position at 37˚C with picric acid indicator papers place inside the lids. After incubation observed the filter paper changes from yellow to orange brown indicates the presence of cyanide.

Results and Discussion

In the present investigation suggested that the screening of potassium solubilizing bacteria (PSB) were analysed from the PSB_1 to PSB_6 strains of *Rhizobium* with growth diameter was 6, 6, 7, 4, 4 and 7mm measured and zone of diameter 3, 3, 4, 0.2 and 8mm with the ratio of 0.5, 0.5, 0.7, 0.5 and 4.0% were analysed a wide assay of rhizosphere of *Vinica faba* of bacteria modulating process and vital role of potassium solubiling activity (Table 1 and Fig.1).

Among the K bearing silicate minerals mica was found to solubilize readily than other minerals (Sugumaran and Janarthanam, 2007; Mikhailouskaya and Tehernysh, 2005) [16, 11]. Similar results to this study were also reported by Archana *et al.* (2008) [3] on KSB, who found that many species of *Bacillus* and *Pseudomonas* were able to solubilizing 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 this study are in agreement with other investigators who reported that *Bacillus megaterium* (Hu and Boyer, 1996) [8] and *B. mucilaginosus* (Biswas and Basak, 2014) [4] were capable of solubilizing mica in appreciable amounts. Archana *et al.* (2008) [3] reported that KSB *Bacillus sp*. solubilizing 44.49 µg/ml mica in liquid medium.

In the current research stated that the strain in PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 was 102.5, 98.9, 72.7, 33.4, 121.2 and 176.4 mg/ml of mole acetic acid production recorded respectively. Whereas Gibberellic acid from the strain of PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 was 108, 78, 89, 56, 121 and 159 µg/ml of production from *Rhizobium* sp. recorded 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) [18, 19]. 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) [7, 12] 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 stated that the products of siderophores was 1.6, 3.9, 8.7, 1.4, 0.8 and 0.4 µg/ml with PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 strain of *Rhizobium* have promoting plant growth promoting Rhizobacteria activities represented respectively. On the other hand, the PSB_1, PSB_2, PSB_3, PSB_4, PSB_5 and PSB_6 of *Rhizobium* sp. strains was produced the HCN from the role of physiological functions. From this study, *Vinica faba* plant rhizosphere with six plant growth promoting Rhizobacteria (PGPR) from the soil which could be directly attributed to the beneficial effects from biological nitrogen fixation and phytohormone production and indiscreetly to phosphate and potassium solubility iron siderosphere and hydrolyzing enzyme production for the growth of the *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). Found that potassium release from the minerals was affected by pH, oxygen and the bacterial strains used. The efficiency of potassium solubilization by different bacteria was found to vary with the nature of potassium bearing minerals and aerobic conditions. 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.

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

S. No	Isolates	Growth diameter (mm)	Zone of 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	4.0

Table 2: Estimation of plant growth hormone from *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

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

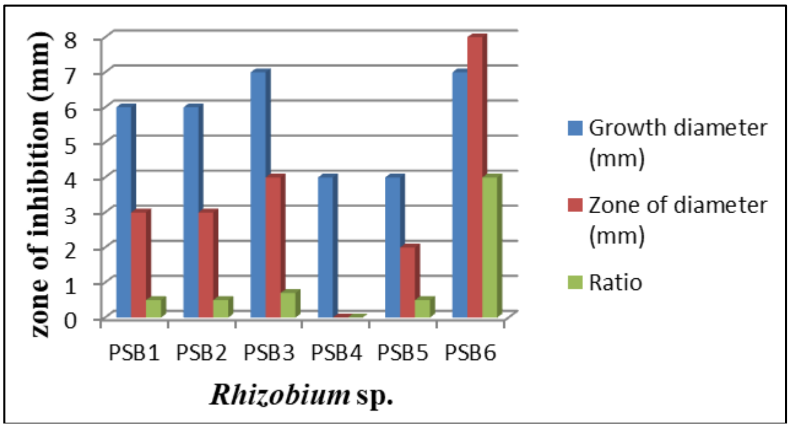

Fig 1: Screening of Potassium solubilizing for *Rhizobium* sp by plate assay

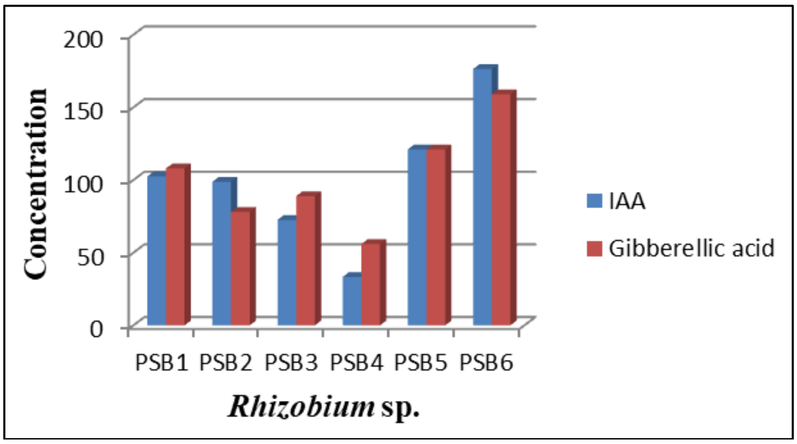

Fig 2: Estimation of plant growth hormone from *Rhizobium* sp.

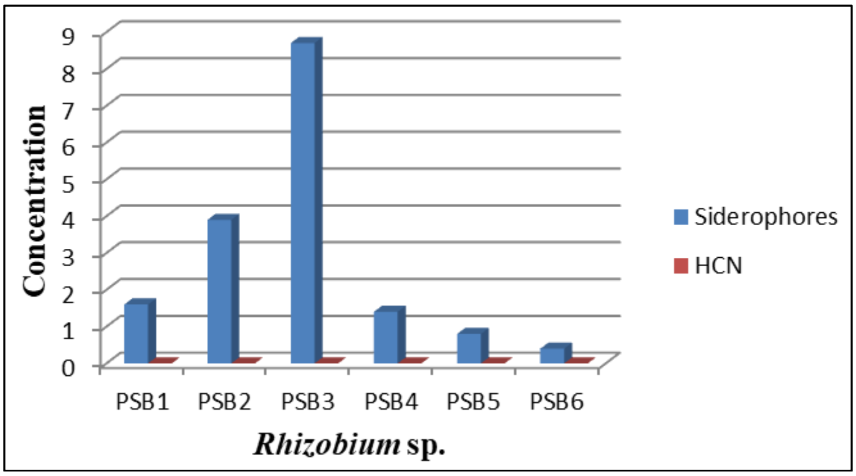

Fig 3: Analysis of Siderophore production and HCN production from *Rhizobium* sp.

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