Isolation and Characterization of Phosphate Solubilizing Bacteria from the Soils of Sikkim, India

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Article History

Manuscript No. c75
Received in 29th August, 2012
Received in revised form 5th March, 2013
Accepted in final form 4th June, 2013

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Keywords

Phosphate solubilizing bacteria, *Bacillus*, *Pseudomonas*, Sikkim

Abstract

The native population of phosphate solubilizing bacteria (PSB) was studied in the rhizosphere of maize, rice, ginger and large cardamom grown in different regions of Sikkim. A total of 26 best PSB were isolated based upon their solubilization on solid media from 104 rhizospheric samples collected from three agroclimatic zones. The population density of PSB showed large variations (2-36×10^6 cfu g^{-1}) and biodiversity within the crop and place of sampling. Phosphate solubilization of these isolates varied from 46 to 160% and 30.2 to 203.7 mg L^{-1} in solid and liquid Pikovskaya's medium, respectively. All the isolates were able to solubilize aluminium and iron phosphate significantly. Among the three phosphate sources all the isolates solubilized $Ca_3(PO_4)_2$ to a greater extent than $AlPO_4$ and $FePO_4$ with $AlPO_4$ exhibiting poor solubilization. The antibiotic resistance pattern showed large variations among the isolates and most of the isolates were resistant to ampicillin, methicillin and metronidazole. The highest PSB number and greatest variability were found in the rhizosphere of rice. Based on the morphological and biochemical analysis the isolates were clustered under the genera *Bacillus*, *Pseudomonas*, *Micrococcus* and *Delftia*. Among the four genera, *Bacillus* was the most predominant PSB found in all of soils tested.

1. Introduction

Phosphorus is the second most important plant nutrient after nitrogen (Donahue et al., 1990). Of the total soil phosphate, only 1-5% is in a soluble, plant available form and the rest became unavailable due to its fixation in soil as insoluble phosphates of iron, aluminum and calcium (Molla and Chowdhury, 1984). Hence, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Certain soil microorganisms like phosphate solubilizing bacteria (PSB) have inherent capacity to dissolve part of the fixed phosphorus and make it available to the crop by secreting low molecular weight organic acids and enzymes (Kucey et al., 1989; Rodriguez and Fraga, 1999; Tilak et al., 2005; Khan et al., 2009). In addition to P-solubilization, few bacteria are capable of producing plant growth hormones, siderophores and have antifungal activities (Weller and Thomashow, 1994; Ponmurugan and Gopi, 2006; Mehta et al., 2010). Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers. Thus, isolation of superior PSB strains for use is to be given immense importance in biofertilizer research.

Several bacterial genera including *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Enterobacter, Azotobacter, Agrobacterium, Flavobacterium* and *Micrococcus*, reported to solubilize phosphorous, were mostly isolated from the temperate countries under neutral and alkaline conditions (Jorquera et al., 2008; Chang and Yang, 2009; Dastager et al., 2010). However, little information is available on the availability of bacteria to solubilize phosphate under acidic and subtropical soil conditions (Chen et al., 2006; Fankem et al., 2006). In the north-eastern region of India, having rich biodiversity with different agro ecological conditions, there is a possibility of presence of various strains of PSB. The present study was therefore, aimed at looking for the biodiversity of PSB in the rhizospher soil of major crops of Sikkim i.e., maize (*Zea mays*), rice (*Oryza sativa*), ginger (*Zingiber officinale*) and large cardamom (*Amomum subulatum*), grown at different regions. It will help in the selection of superior PSB strains and dominant types of bacteria involved in P-solubilization.

2. Materials and Methods

2.1. Sample collection

Sikkim covers a total land area of 7096 sq. Km. between 27°

04 - 28° 07 N and 88° 00 E-88° 5 E. The thumb-shaped state of Sikkim is characterized by wholly mountainous terrain, with the elevation ranging from 280 meters to 8,585 meters. Sikkim is geographically diverse, owing to its location on the Himalayas and has four agroclimatic zones viz., sub-tropical high humid (approximate altitude<1000 m, average air temperature>20° C), temperate humid (approximate altitude 1000- 2000 m, average air temperature 10- 20° C), sub-alpine low humid (approximate altitude 2000- 3000 m, average air temperature<10° C) and alpine dry zone (approximate altitude >3000 m, average air temperature<10° C). The investigation was carried out in three agroclimatic zones of Sikkim (as the alpine dry zone does not contain any crop vegetation under study). A total of 104 soil samples were collected from the rhizosphere of maize (17), rice (38), ginger (24) and large cardamom (25) crops grown in different regions of Sikkim. The soil samples approximately 400-500 g were obtained from different field crops and stored in plastic bags at low (4°C) temperature until further processing. These soils were air dried, crushed to pass through 2 mm sieve and thoroughly mixed to represent one composite sample.

2.2. Isolation and identification of PSB

Ten grams of soil sample was suspended in 90 ml of sterile distilled water to obtain 10^{-1} dilution. Serial dilutions were prepared by mixing 1 ml of the suspension made into 9 ml of the sterile distilled water, until the 10^{-7} dilution was obtained. Three aliquots of 0.1 ml from each dilution was plated on Pikovskaya's medium (10 g glucose, 5 g tricalcium phosphate, 0.5 g ammonium sulphate, 0.2 g potassium sulphate, 0.1 g magnesium sulphate, 0.5 g yeast extract, 0.002 g manganese sulphate and 0.002 g ferrous sulphate in 1L distil water; Pikovskaya, 1948) and NBRI-BPB medium (10 g glucose, 5 g tricalcium phosphate, 5 g magnesium chloride, 0.25 g magnesium sulphate, 0.2 g potassium chloride, 0.1 g ammonium sulphate and 0.025 g bromophenol blue in 1L distil water; Nautiyal, 1999) with 2% agar powder. The plates were incubated at 30±1 °C for 5 days. The bacterial colonies showing halo zone on Pikovskaya's plate or yellow colored halo zones around the colonies on NBRI-BPB plates were selected as phosphate solubilizers. The colonies were purified and maintained on Pikovskaya's slants for further study. All the isolates were classified on the basis of colony characteristics such as the size, color, shape and texture of each isolate. A standard PSB *Pseudomonas striata* was obtained from division of microbiology, Indian Agricultural research Institute, New Delhi, India was used as a reference strain.

2.3. Identification and characterization of PSB isolates

All the PSB isolates were identified and characterized by subjecting the isolates to several biochemical tests, viz., indole production, methyl red, Voges Proskauer test, citrate production, nitrate reduction, urease test, H_2S production, catalase test, oxidase test, gelatin liquefaction, utilization and acid production from glucose, sucrose and lactose as prescribed in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1994; Sharma, 2007).

2.4. Determination of P-solubilization activity of PSB isolates

Phosphate-solubilization by each PSB isolate was assessed on solid as well as in liquid culture conditions using NBRI-BPB plates and Pikovskaya's medium, respectively. The broth culture of each isolate was spotted on NBRI-BPB plates for determination of P-solubilization efficiency. The plates were incubated at 30±1 °C for 3 days and the diameter of the colony as well as the halo zone was measured. P-solubilization efficiency (PSE) was calculated as:

PSE (%)=[(Z–C)÷C]×100; where, Z=halo zone diameter, C=colony diameter

P-solubilization in Pikovskaya's liquid medium was analyzed under stationary conditions by growing the different isolates with known amount of tricalcium phosphate as a substrate. For testing the P-solubilization of ferric and aluminum phosphate the same Pikovskaya's medium was used but instead of $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$ were used, respectively. Fifty ml of liquid medium was inoculated with 2 ml broth of 24 h old culture from a single colony and uninoculated flasks were kept as control. The flasks were incubated at 30±1ºC. At each sampling date, the contents were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for soluble P-content following the method described by Murphy and Riley (1962). A change in pH of the medium due to growth of PSB was measured with a pH meter after each sampling.

2.6. Antibiotic resistance of PSB

The antibiotic resistance (AR) pattern of all PSB isolates was examined using Nutrient agar medium. A single colony of each PSB isolate was inoculated into 5 ml of nutrient broth and incubated at 30±1ºC for 24 h. The culture broth was spread evenly on the nutrient agar plates with the help of a spreader. 19 antibiotic discs (HiMedia) with different concentrations were spotted on the nutrient agar plates loaded with the bacterial isolates and the plates were incubated overnight. The halo zone formation was examined for all antibiotic discs with respect to the bacterial isolates.

2.7. Statistical analysis

All experiments were performed in triplicate to check the reproducibility and the results were expressed as mean values. Results were statistically analysed by Duncan's new multiple range test.

3. Results and Discussion

3.1. Isolation of PSB from the rhizosphere soil

Several PSB were isolated from the rhizospheric soil of maize, rice, ginger and large cardamom crops, but only 26 isolates (maize-7, rice-8, ginger-6, large cardamom-5) with having high P-solubilization efficiency on plate and showing high morphological variability were selected for subsequent studies. The pH of the collected soil samples were found in the range of 4.5 to 6.5. The population density of PSB with respect to different field crops soils are presented in Table 1. PSB count varied from 2-36×10^6 cfu g^{-1} of soil in the rhizosphere of different crops. This variation in the population of PSB might be attributed to many soil factors such as soil nutrient, pH, moisture and organic matter content (Vikram et al., 2007). The PSB population is found to be higher in the rice field and healthy cardamom field. It was observed that irrigated crops showed higher PSB population than dry land crops which affirms the earlier findings of Vikram et al. (2007).

Table 1: Population density of PSB in rhizosphere soil of different field crops

Name of field crop	Designation of strain	Population density (cfu g^{-1} soil)
Maize	M14	2×10^6
Maize	M16	12×10^6
Maize	M28	4×10^6
Maize	M51	2×10^6
Maize	M61	3×10^6
Maize	M71	8×10^6
Maize	M510	3×10^6
Rice	R31	36×10^6
Rice	R33	5×10^6
Rice	R42	11×10^6
Rice	R62	4×10^6
Rice	R63	7×10^6
Rice	R71	10×10^6
Rice	R72	2×10^6
Rice	R81	5×10^6
Ginger	G14	4×10^6
Ginger	G15	14×10^6
Ginger	G21	2×10^6
Ginger	G41	2×10^6
Ginger	G42	5×10^6
Ginger	G49	2×10^6
Large cardamom	C22	8×10^6
Large cardamom	C31	31×10^6
Large cardamom	C41	3×10^6
Large cardamom	C51	5×10^6
Large cardamom	C61	3×10^6

3.2. Inorganic phosphate solubilization by PSB on solid and liquid medium

Two different media Pikovskaya's and NBRI-BPB were used for isolation of PSB. For some isolates a distinct clear halo zone was not observed in the Pikovskaya's solid medium whereas, they are able to solubilize phosphate in the liquid medium. This result corroborates with the earlier reports of Chung et al. (2005). On the otherhand a contrasting yellow zone was visible around the colonies on NBRI-BPB plates for the same isolate thus agreeing with the view of Mehta and Nautiyal (2001) that NBRI-BPB medium is an efficient method for screening of PSB. So NBRI-BPB medium was used for isolation of PSB from the soil samples. On the otherhand growth of the isolates were found slower in NBRI-BPB medium as compared to Pikovskaya's medium. So Pikovskaya's liquid medium was used for quantification of phosphate solubilization by each isolates.

The halo zone of P-solubilization on NBRI-BPB plates by some PSB isolates are clearly visible in Figure 1. Phosphate solubilization pattern of the PSB isolates in Pikovskaya's liquid medium was studied under stationary condition. Samples were withdrawn at regular intervals up to 17 days and the P-solubilization in the medium was analyzed. Maximum solubilization was observed between 9-14 days in all the PSB isolates. Figure 2 shows the P-solubilization of few isolates in liquid Pikovskaya medium at regular interval of time and compared with the known strain of *Pseudomonas striata*. The isolates with high P-solubilization efficiency (R63 and C61) showed a gradual increase in P-solubilization from the day of incubation and maximum P-solubilization was observed on day 12. Similar pattern was also observed in cash of *P. striata*. However, the isolates with lower P-solubilization efficiency (C31 and G14) showed a sudden increase in P-solubilization up to day 4 followed by a slower solubilization rate (Figure 2). For aluminum and iron phosphate maximum P-solubilization was observed on day 10 of incubation. This shows that the rate of phosphate solubilization is species specific and depends on the phosphate source.

The phosphate solubilizing efficiencies of all the PSB isolates in liquid medium containing tricalcium phosphate, aluminum phosphate and iron phosphate one at a time as the sole phosphate source were summarized in Table 2. It was clearly evident that the P-solubilized by all the isolates were significantly different from that of the control (p<0.05), indicating that the tested isolates had effectively converted the inorganic insoluble phosphate into soluble form. Among the isolates, M61 was found as the best in solubilizing calcium phosphate (203.7 mg L^{-1}) while the lowest was shown by R33

Table 2: Solubilization of inorganic phosphates by the PSB isolates in liquid medium

PSB isolates	Medium with $Ca_3(PO_4)$		Medium with $Al(PO_4)$		Medium with $Fe(PO_4)$	
	Pi (mg L^{-1})	pH	Pi (mg L^{-1})	pH	Pi (mg L^{-1})	pH
Control	17.2^{a}	7.0	18.9^{a}	6.9	19.7^{a}	6.8
M14	184.0^{m}	4.7	39.3^{d}	4.9	65.8^{cd}	4.5
M16	135.6^{i}	5.3	37.2^{d}	5.1	66.3^{cd}	4.5
M28	55.2^{d}	4.9	36.6^{cd}	5.2	60.6^{bc}	5.4
M51	182.3^{m}	4.8	48.7^{ef}	4.9	78.5^{gh}	5.5
M61	203.7^{o}	4.7	42.4^{de}	5.0	73.5^{efg}	5.6
M71	103.4^{g}	5.1	46.6^{d}	5.1	78.1^{gh}	5.5
M510	158.3^{k}	4.7	61.3^{g}	5.4	100.7^{j}	5.3
G14	72.5^{ef}	5.1	32.3^{bc}	5.2	59.8^{bc}	4.9
G15	64.3^{e}	5.2	34.5^{cd}	5.1	67.6^{cde}	5.3
G21	164.4^{kl}	4.6	50.8^{ef}	5.3	68.5^{cde}	5.8
G41	76.4^{f}	5.1	32.9^{c}	5.2	61.1^{bc}	5.7
G42	69.8^{ef}	5.2	35.7^{cd}	5.1	64.5^{c}	5.5
G49	193.5^{n}	4.7	39.7^{cd}	5.0	64.7^{c}	5.3
R31	169.1^{l}	5.2	51.2^{ef}	5.2	71.2^{def}	5.2
R33	30.2^{b}	6.3	36.5^{cd}	5.3	60.3^{bc}	5.4
R42	115.6^{h}	6.4	38.9^{cd}	5.0	57.6^{b}	5.4
R62	66.7^{e}	6.2	43.7^{de}	5.2	72.1^{ef}	5.8
R63	157.3^{k}	4.7	46.1^{ef}	5.3	85.4^{i}	5.1
R71	144.8^{j}	4.7	48.2^{ef}	5.4	73.3^{efg}	5.2
R72	55.6^{d}	5.2	37.6^{cd}	5.5	58.2^{b}	5.4
R81	34.6^{bc}	5.2	29.3^{b}	5.1	68.6^{cd}	4.8
C22	71.2^{ef}	5.1	27.5^{b}	5.0	65.1^{cd}	4.7
C31	42.1^{c}	5.4	31.3^{bc}	5.2	69.1^{cd}	4.8
C41	165.7^{kl}	4.6	30.2^{bc}	4.8	56.3^{b}	5.0
C51	109.9^{gh}	5.1	33.5^{c}	5.0	63.2^{c}	4.9
C61	196.2^{no}	4.6	46.1^{ef}	5.3	75.2^{fg}	5.1
P. striata	193.2^{n}	4.6	51.9^{f}	5.2	82.0^{hi}	5.1

P-solubilization in $Ca_3(PO_4)_2$ of 12th day culture and in $Al(PO_4)$, $Fe(PO_4)$ of 10th day culture Values are means of three independent observations. SE values ranges from 0.42 to 3.91. Values in the column superscripted by different letters are significantly (p<0.05) different from each other (Duncan's new multiple range test). Separate analysis was done for each column.

(30.2 mg L^{-1}). The isolates C22 and C41 showed the least P-solubilization for aluminum and iron phosphate, respectively whereas, M510 showed maximum solubilization for both cashes i.e. up to 61.3 mg L^{-1} and 103.4 mg L^{-1} respectively, for $AlPO_4$ and $FePO_4$. The results also indicated that M510 is a better $AlPO_4$ and $FePO_4$ solubilizer than that of the known strain *P. striata*. Among the three phosphate sources all the isolates solubilized $Ca_3(PO_4)_2$ to a greater extent than $AlPO_4$

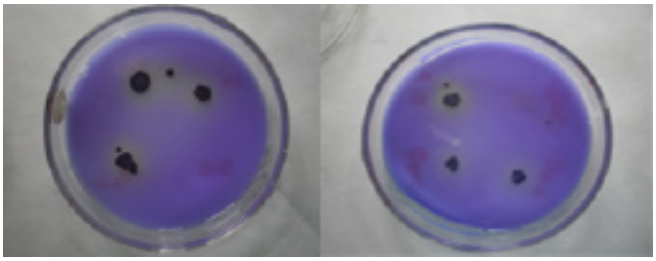

Figure 1: Zone of P-solubilization by selected PSB isolates on NBRI-BPB agar

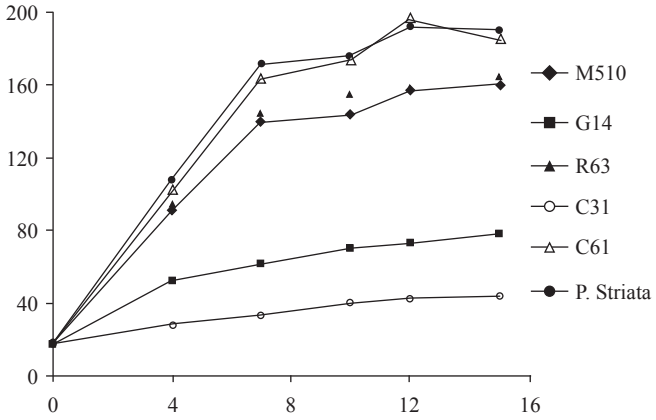

Figure 2. P-solubilization pattern of few isolates in Pikovskaya liquid medium

and $FePO_4$ with $AlPO_4$ exhibiting poor solubilization (Table 2). This is in well agreement with Chung et al. (2005) where PSB isolates from Korea showed maximun P-solubilization in media containing $Ca_3(PO_4)_2$ followed by $FePO_4$ and lowest by $AlPO_4$, but contradicts Fankem et al. (2006) where PSB isolates from Cameroon showed poor solubility to ferric phosphate.

A decrease in pH of the medium was observed for all the tested isolates as compared to the control (Table 2). This might be due to the secretion of organic acids by PSB. With similar pH value different phosphate solubilization efficiency was also observed. This might be due to the quality and quantity of organic acids produced differs in different organisms (Illmer and Schinner, 1992; Rodriguez and Fraga, 1999).

3.3. Characterization of PSB isolates

All the 26 PSB isolated from the rhizospheric soil of the four crops were characterized morphologically as well as on the basis of their biochemical analysis and antibiotic resistance pattern.

3.3.1. On the basis of morphological characteristics

The morphological characteristics of the PSB isolates are shown in Table 3. The colony morphology of PSB isolates collected from different locations showed variations with respect to most of the colony characteristics (colour, size, shape and texture). Most of the PSB isolates were white or

off-white, but a few showed yellow color pigmentation. The size of the colonies varied from very small to large with round or irregular shape and the texture of the colonies was dry, semi-dry or gummy. Gram's staining of isolates revealed both Gram negative and Gram positive small rods and cocci in the rhizospheric soil.

3.3.2. On the basis of biochemical analysis

All the PSB isolates were characterized biochemically by carrying out 16 biochemical tests and are summarized in Table 3. The colonies having morphological similarities differ greatly on biochemical analysis. Both positive and negative results were observed for citrate utilization, nitrate reduction, methyl red, Voges Proskauer, catalase and oxidase tests. All most all isolates were found positive for urease test. All the isolates were found negative for indole production and hydrogen sulphide production. Except one isolate, all were negative for gelatin liquefaction. Most of the isolates were able to ferment glucose and sucrose whereas, only 6 isolates could ferment lactose. Table 3 showed that the most dominant phosphate solubilizing bacteria found were Gram positive aerobic spore forming motile bacteria. Identification of this group showed that it belongs to the genera *Bacillus*. On the basis of morphological and biochemical analysis the other bacteria were tentatively grouped under the genus *Pseudomonas*, *Micrococcus* and *Delftia*.

3.3.3. On the basis of P-solubilization on solid and liquid Pikovskaya's medium

The PSB isolates were grouped on the basis of PSE% and P-solubilization to correlate their solubilizing capability with crop field and region of isolation (Table 4). All the 26 isolates were categorized in to 4 groups on the basis of PSE. None of the isolates from maize, rice and large cardamom showed PSE<50%, while only one isolate from ginger exhibited PSE

Table 3: Morphological and biochemical characterization of PSB isolates from different field crops

Name of isolate	Gram staining	Shape	Colony color	Spore staining	Motility	Indole	MR	VP	Citrate	Nitrate	Urease	HS production	Catalase	Oxidase	Gelatin liquefaction	Carbohydrate utilization		
																Sucrose	Lactose	Glucose
M14	+	cocci	white	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+
M16	+	cocci	white	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+
M28	+	rod	white	+	+	-	-	+	+	+	+	-	+	-	-	+	-	+
M51	+	rod	white	+	+	-	-	-	+	-	+	-	+	-	-	-	-	+
M61	+	rod	white	+	+	-	+	-	+	-	+	-	+	-	-	+	-	+
M71	+	rod	white	+	+	-	+	+	+	+	+	-	+	+	-	+	-	+
M510	+	rod	white	+	+	-	+	-	+	+	+	-	+	+	-	-	-	+
G14	+	rod	white	+	+	-	-	+	+	+	+	-	+	-	-	+	-	+
G15	+	rod	white	+	+	-	-	+	+	+	+	-	+	-	-	+	-	+
G21	+	rod	white	+	+	-	-	-	+	+	+	-	+	+	-	+	-	+
G41	+	rod	white	+	+	-	+	-	+		+	-	+	-	-	+	+	+
G42	+	rod	white	+	+	-	-	+	+	+	+	-	+	-	-	+	-	+
G49	+	cocci	yellow	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+
R31	+	rod	white	+	+	-	-	-	+	+	+	-	+	+	-	-	-	-
R33	+	rod	white	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+
R42	+	rod	white	+	+	-	-	-	+	-	+	-	+	+	-	-	-	-
R62	-	rod	yellow	-	+	-	-	-	+	-	+	-	-	+	-	-	-	+
R63	+	cocci	yellow	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+
R71	-	rod	white	-	+	-	-	-	+	-	+	-	+	+	-	-	-	-
R72	+	cocci	yellow	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+
R81	+	rod	white	+	+	-	+	-	+	-	+	-	+	+	-	+	-	+
C22	+	rod	white	+	+	-	-	+	+	+	+	-	+	+	-	+	-	+
C31	+	rod	white	+	+	-	-	+	+	+	+	-	+	+	-	+	-	+
C41	+	cocci	white	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+
C51	+	rod	white	+	+	-	-	-	+	+	+	-	+	+	-	+	-	+
C61	-	rod	white	-	+	-	-	-	+	-	-	-	+	+	-	-	-	-

in this range. Three isolates from maize showed PSE between 51-100% and four exhibited PSE between 101-150%. Among 6 isolates of ginger one shows PSE between 51-100% and 4 between 101-150%. Three isolates from rice showed PSE between 51-100% and four exhibited PSE between 101-150% and only one isolate showed PSE>150%. Among large cardamom isolates one showed PSE between 51-100% and four exhibited PSE in the range of 101-150%. None of the isolates from maize, ginger and cardamom exhibited PSE>150%. On the whole maximum number of isolates (16) showed PSE% in the range of 101-150%, whereas, the groups of PSE%<50% and>150% were represented by a single isolates.

On the basis of P-solubilization in liquid pikovskaya's medium, PSB were again categorized in to 4 groups (Table 4). 12% isolates fell in to the first group showing P-solubilization< 50 mg L^{-1}, whereas, the second group (51-100 mg L^{-1}) and third group (101-150 mg L^{-1}) consisted of 31% and 19%, respectively. The fourth group showing maximum P-solubilization (>150 mg L^{-1}) contained 10 isolates and consisted of 38% of the total. The isolates from maize were distributed in three groups showing P-solubilization in between 51-200 mg L^{-1}. The isolates from rice and large cardamom were distributed to all four groups whereas, the isolates from ginger showed P-solubilization in the range of 51-100 and >150 mg L^{-1}. The results showed a wide range of variations in P-solubilization efficiency of the PSB isolates irrespective of the crop field and region of Sikkim from where it was isolated. There was no correlation drown between P-solubilization efficiency on solid and liquid medium. This is quite in tune with the earlier reports of Ponmurugan and Gopi (2006) and Kundu et al. (2009).

Table 4: Grouping of PSB isolates on the basis of P-solubilization on solid and in liquid Pikovskaya's media

Group of isolates	P-solubilization efficiency (PSE%)	Maize (7)	Ginger (6)	Rice (8)	Cardamom (5)	Total (26)
I	0-50	0 (0)	1 (17)	0 (0)	0 (0)	1 (4)
II	51-100	3 (43)	1 (17)	3 (38)	1 (20)	8 (31)
III	101-150	4 (57)	4 (67)	4 (50)	4 (80)	16 (62)
IV	>150	0 (0)	0 (0)	1 (13)	0 (0)	1 (4)
Group of isolates	*P-solubilization efficiency (mg L^{-1})	Maize (7)	Ginger (6)	Rice (8)	Cardamom (5)	Total (26)
I	0-50	0 (0)	0 (0)	2 (25)	1 (20)	3 (12)
II	51-100	1 (14)	4 (67)	2 (25)	1 (20)	8 (31)
III	101-150	2 (29)	0 (0)	2 (25)	1 (20)	5 (19)
IV	>150	4 (57)	2 (33)	2 (25)	2 (40)	10 (38)

*P-solubilization value of 12th day culture. Values in parentheses represent percentage of the total isolates of the respective crops.

3.3.4. On the basis of the antibiotic resistance pattern

Antibiotic resistance reflects the presence of genes having the capability to detoxify the antibiotics or to modify the cell membrane so as not to allow the antibiotics to reach the site of action. Hence, the antibiotic resistance pattern was used as a criterion to know the diversity among the 26 isolates using nineteen antibiotics. All the 26 isolates (Table 5), irrespective of the host plant, were resistant to methicillin (100%), followed by ampicillin (89%) and a great majority were also resistant to metronidazole (63%). The isolates were comparatively less resistant to ampicillin or cloxacillin (27%), co-trimoxazole (19%) and furozone (19%). PSB isolates showed least resistance of about 15% to the antibiotics amoxycillin, furazolidone and nitrofuranloin. The PSB isolates were found resistant to either A-M or M-Mt whereas, all 26 isolates were found to be sensitive to ciprofloxacin, chloramphenicol, doxycycline hydrochloride, erythromycin, enrofloxacin, gentamicin, kanamycin, oxytetracycline, streptomycin and tetracycline (data not shown). This shows that the PSB isolates were mostly resistant to A, M and Mt. However, according to Kundu et al. (2009) the PSB isolated from Haryana, India were

Table 5: Antibiotic resistance of PSB isolates from rhizosphere of different crops

Antibiotic	Concentration (mcg)	Maize (7)	Ginger (6)	Rice (8)	Cardamom (5)	Total (26)
Ampicillin (A)	25	7 (100)	6 (100)	5 (63)	5 (100)	23 (89)
Amoxycillin (Am)	30	1 (14)	1 (17)	1 (13)	1 (20)	4 (15)
Ampicillin or Cloxacillin(Ax)	10	3 (43)	1 (17)	2 (25)	1 (20)	7 (27)
Co-trimoxazole (Co)	25	0 (0)	0 (0)	3 (38)	2 (40)	5 (19)
Furazolidone (Fr)	50	0 (0)	1 (17)	2 (25)	1 (20)	4 (15)
Furozone (Fx)	100	0 (0)	1 (17)	3 (38)	1 (20)	5 (19)
Metronidazole (Mt)	5	5 (71)	2 (33)	6 (75)	3 (60)	16 (62)
Methicillin (M)	30	7 (100)	6 (100)	8 (100)	5 (100)	26 (100)
Nitrofuranloin (Nf)	300	0 (0)	1 (17)	2 (25)	1 (20)	4 (15)

Values in parentheses show percentage of isolates resistant to antibiotics.

mostly resistant to streptomycin, ampicillin and penicillin. The AR pattern of different isolates is not confined to any particular crop or region of Sikkim. This shows the diversity of soil bacteria varies from place to place and in the rhizosphere of different crops.

4. Conclusion

The results in the present study reveal that a large and diverse repository of PSB exists in the rhizosphere of maize, rice, ginger and large cardamom in the Sikkim state. Although the PSB were widely distributed throughout the state the higher diversity and population density was evident in the rice rhizosphere. Variations were observed within the isolates of each crop on the basis of morphology, biochemical analysis, P-solubilization efficiency and antibiotic resistance pattern. Among the 26 isolates, spore forming *Bacillus* was the most predominant PSB found in the acidic soils of Sikkim. The strain M510 was found as a better aluminum and iron phosphate solubilizer. Collection of this diverse type of PSB can help in the selection of highly efficient strains and further studies are required to prove the nature of these isolates to harness their potential as bioinoculants in agriculture.

5. Acknowledgements

Financial support from Council of Scientific and Industrial Research (CSIR), New Delhi, India is thankfully acknowledged.

6. References

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