Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana

B. S. Kundu · Kiran Nehra · R. Yadav · M. Tomar

Abstract   The native population of phosphate solubilizing bacteria (PSB) was studied in the rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. A total of 193 PSB were isolated from 245 rhizospheric samples collected from south-west and north-east zones. The PSB count showed large variations ($3–67 \times 10^5$cfu/g) and biodiversity within the crop and place of sampling. Using biochemical analysis, the isolates were tentatively identified as belonging to four genera, *Pseudomonas*, *Aeromonas*, *Klebsiella* and *Enterobacter*. Phosphate solubilization of these isolates varied from 5.9 to 123.8% and 2.2 to 227.2 µg/ml in solid and liquid Pikovskaya's medium, respectively. Based on their morphological traits, all the isolates were placed into 20 groups, majority of them falling in the group having white, round and gummy colonies, irrespective of the crop or the region. The intrinsic antibiotic resistance pattern showed large variations among the isolates and most of the isolates were resistant to streptomycin, ampicillin and penicillin. The highest PSB number and greatest variability were found in the rhizosphere of chickpea, followed by wheat and then mustard.

Introduction

Phosphorous is the second major nutrient for plants, however, it is the least soluble in soil. The total phosphorous in the soil ranges from 0.01 to 0.2 per cent but only a small amount of it is available to the plants. Phosphorous exists in nature in a variety of organic and inorganic forms which are insoluble to very poorly soluble [1]. Plants acquire phosphorous from soil solution as phosphate anions which are extremely reactive and get immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of the soil [2, 3]. Several soil microorganisms known as phosphate solubilizing bacteria (PSB) have the ability to solubilize insoluble mineral phosphate by producing various organic acids, siderophores, mineral acids, protons, humic substances, CO_2 and H_2S [4, 5]. This results in acidification of the surrounding soil, releasing soluble orthophosphate ions ($H_2PO_4^{-1}$, HPO_3^{-2} and PO_4^{-3}) which can be readily taken up by plants.

A large number of PSB have been isolated from the rhizosphere of several crops [6, 7] and these constitute about 20 to 40% of the culturable population of soil microorganisms. The important genera of PSB include *Achromobacter*, *Aerobacter*, *Alkaligenes*, *Bacillus*, *Pseudomonas*, *Serratia* and *Xanthomonas* [8, 9]. In addition, certain fungi known as phosphate solubilizing fungi (PSF) have also been shown to solubilize insoluble phosphate [10]. These organisms have been in use as biofertilizers since 1962 [11] and are gaining importance in the recent years due to their vital role in maintaining the soil nutrient status and structure. Increase

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in the yield of crops like cereals, legumes, oils, fibres and vegetables by inoculation with PSB has been reported [12–17].

Extensive studies have been conducted on isolation of PSB and their role in crop production and a few on the abundance and distribution of PSB in some regions [18], but the information available on their diversity is still very scanty. A diverse population of organisms is normally more resilient to stress and better capable of adapting to environmental changes. Bacterial diversity increases soil quality by affecting soil agglomeration and increasing soil fertility and enhancing plant health through nutrient recycling [19]. Also, a continued exploration of the natural biodiversity of soil microorganisms, and the optimization and manipulation of microbial interactions in the rhizosphere of crops represents a prerequisite step to develop more efficient microbial inoculants. It is thus imperative to conserve and characterize the variability of various agriculturally important microorganisms for their optimal utilization in the development of future technologies and also for their use as biofertilizers, biopesticides, growth promoters and microbial community developers. The present study was therefore, aimed at looking for the biodiversity of PSB in the rhizosphere of important crops like chickpea (*Cicer arietinum*), mustard (*Brassica campestris*) and wheat (*Triticum aestivum*) grown in different regions of Haryana. A diverse inventory of PSB will certainly strengthen the crop improvement strategy with PSB as bioinoculants. It will help in the selection of dominant types of bacteria involved in P-solubilization.

Materials and methods

Isolation and characterization of PSB from rhizosphere of chickpea, mustard and wheat: PSB were isolated from 245 samples collected from the rhizosphere of chickpea (55), mustard (99) and wheat (91) grown at different locations of south-west and north-east zones of Haryana. Intact roots along with adhered soil from all the three host plants were collected and stored in plastic bags at low (4ºC) temperature until further processing. PSB were isolated by dilution plating using appropriate dilutions on Pikovskaya's (PVK) medium [20]. The probable isolates showing a discrete halo zone around colonies (as a result of utilization of tri-calcium phosphate present in the medium) were assumed to be phosphate solubilizers. These colonies were restreaked for purification and P-solubilization prior to transfer on PVK slants for maintenance. The total bacterial and PSB counts of each soil sample were recorded by plating on solid medium plates. All the isolates were classified on the basis of colony characteristics such as the size, colour, shape and texture of each isolate.

Identification and characterization of PSB isolates: All the PSB isolates were identified and characterized by subjecting the isolates to several biochemical tests, viz., starch hydrolysis, nitrate reduction, indole production, methyl red, Voges Proskauer test, citrate production from glucose, gelatin liquefaction, oxidase test, H_2S production, utilization and acid production from glucose, galactose, fructose, mannitol, sucrose, maltose, cellobiose, inositol, lactose and xylose as prescribed in Bergey's Manual of Systematic Bacteriology [21, 22]. All the twenty biochemical characters for the PSB were analyzed using UPGMA software programme.

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 PVK media. The culture broth having approximately 10^8cfu/ml was spotted on PVK plates for determination of the P-solubilization efficiency. The plates were incubated at 30 ± 2ºC for two days and the diameter of the colony as well as the halo zone was measured. P-solubilizing efficiency (PSE) was calculated as:

PSE (%) = [Z–C/C] × 100; where, Z = Halo zone diameter, C = Colony diameter

P-solubilization in PVK liquid medium was examined under stationary conditions by growing the different isolates initially in 5 ml nutrient broth and then adding appropriate quantity to 15 ml fresh broth which was incubated for 24 h at 30 ± 2ºC to obtain a population of approximately 10^8 cfu/ml cells. Fifty ml PVK liquid medium was inoculated with 2 ml culture broth and uninoculated flasks were kept as control. The flasks were incubated at 30 ± 2ºC for 2 days and the contents were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for soluble P-content using the method as described by John (1970) [23]. The pH of the supernatant was also recorded.

Intrinsic antibiotic resistance of PSB: The intrinsic antibiotic resistance (IAR) pattern of all PSB isolates was examined using PVK medium supplemented with100 µg/ml of the following antibiotics: kanamycin (Km), ampicillin (Am), streptomycin (Sm), neomycin (Nm), penicillin (Pn) and nalidixic acid (Nx) except chloramphenicol (Cm) and tetracycline (Tc) where the concentration was 50 and 10µg/ml, respectively. A loopful of each PSB isolate was inoculated into 5 ml of nutrient broth and incubated at 30 ± 2ºC for 24 h. An aliquot of 30 µl from each culture was spotted on the PVK plate supplemented with the respective antibiotic and cell growth was examined and compared with the control plate having no antibiotic.

Results and discussion

Isolation of PSB from the rhizosphere of chickpea, mustard and wheat: To determine the diversity of PSB, a total of 245 samples were collected from the rhizosphere of chickpea, mustard and wheat crops grown in different regions of Haryana. Fifty-five samples were collected from the rhizospheric soil of chickpea grown in the south-west zone of Haryana comprising of Bhiwani, Mahendergarh, Jhajjar, Sirsa, Fatehabad and Hisar. The chickpea crop is not grown in the north-east zone; however one sample could be collected from Matlodha (Jind). For the isolation of PSB from mustard and wheat rhizosphere, samples were collected from 99 and 91 different locations respectively, including Bhiwani, Mahendergarh, Sirsa, Fatehabad, Hisar and Jhajjar (south-west zone) and Yamunanagar, Karnal, Kaithal, Jind and Rohtak (north-east zone). Several PSB were isolated from the rhizospheric soil of the three crops, but only 193 isolates (chickpea-76, mustard-68 and wheat-49) showing high morphological variability were finally selected for subsequent studies (Table 1).

The PSB count was about 10 to 100 times lower than the total bacterial count in the samples collected from all the three crops. PSB count from the samples collected from chickpea rhizosphere varied from $9–38 \times 10^5$ cfu/g soil and 67×10^5 cfu/g soil in the south-west zone and north-east zone, respectively. The maximum diversity was found in the samples from Matlodha (Jind), Satnali (Mahendergarh) and Agroha (Hisar) as they showed 5–7 different types of

colonies. The samples collected from mustard rhizosphere showed PSB count varying from $6–13 \times 10^5$ cfu/g soil. The maximum counts of PSB were observed in the soils from Sheelashakti (Bhiwani) and Kalram (Kaithal) and maximum diversity was observed in the soil from Kanjanu (Yamunanagar), Madhuban (Karnal) and Durjanpur (Fatehabad) showing 4–5 different types. The PSB in wheat rhizosphere varied from $4–20 \times 10^5$ cfu/g soil. Maximum PSB count (20×10^5 cfu/g) was observed in the sample from Kalana (Mahendergarh) followed by Raithal (Hisar). Based on colony morphology, four different types of PSB were obtained from Damla (Yamunanagar). The PSB count was highest in the chickpea rhizosphere followed by wheat and mustard.

Identification of the PSB isolates: Identification of the 193 PSB isolates was carried out according to Bergey's Manual of Systematic Bacteriology [21]. A total of 20 biochemical tests were performed, and on the basis of these tests, the isolates were tentatively placed into four genera. Eighty seven isolates belonged to the genus *Pseudomonas*, 27 to *Aeromonas*, 35 to *Klebsiella* and 44 to *Enterobacter*. However, no host specificity was noticed for PSB.

Phosphate solubilization by PSB on solid and liquid PVK medium: The P- solubilization on solid medium was higher in PSB isolated from chickpea and wheat rhizosphere than those isolated from mustard rhizosphere. P-solubilization by isolates from chickpea rhizosphere varied from 6–118% from different locations. The isolate 59°C of chickpea showed maximum solubilization (118%) followed by 58C

Table 1   Total bacterial and PSB ($\times 10^5$ cfu/g) counts from the rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana

Regions	Chickpea				Mustard				Wheat			
	Sample	Bacterial count		PSB types	Sample	Bacterial count		PSB types	Sample	Bacterial count		PSB types
		Total	PSB			Total	PSB			Total	PSB	
South-West Zone												
Bhiwani	09	321	9	4	06	308	13	2	06	176	4	2
Mahendergarh	06	253	18	5	10	352	6	3	05	164	20	2
Sirsa	05	439	38	4	14	184	7	3	14	164	8	3
Fatehabad	02	325	11	4	12	242	12	4	12	155	6	2
Hisar	30	382	10	5	13	212	6	3	11	146	11	2
Jhajjar	02	372	17	3	01	320	7	1	01	170	13	3
Total/Average	54	348	17	3–5	56	270	8	1–4	49	162	10	2–3
North-East Zone												
Yamunanagar	–	–	–	–	11	458	6	5	11	220	12	4
Karnal	–	–	–	–	07	356	12	4	07	262	10	2
Kaithal	–	–	–	–	13	202	13	2	13	162	10	2
Jind	01	530	67	7	10	193	6	3	10	266	11	2
Rohtak	–	–	–	–	02	317	6	2	01	120	3	1
Total/Average	01	530	67	7	43	305	9	2–5	42	206	9	1–4

– = Chickpea not grown in North-East Zone

(114%), 8C (80%), 18C (64%) and 33C (63%). The PSE of the isolates from mustard rhizosphere varied from 6–50%, maximum being observed for 22 M (50%). The PSE of the isolates from wheat rhizosphere varied from 6–71% and maximum PSE was shown by 44W (71%) followed by 19W (69%) and 51W (50%).

In PVK liquid medium, a decrease in pH and increase in soluble P-content was recorded with all the PSB isolates. However, the PSB isolated from the three crops showed large variations in their ability to solubilize phosphorous. Among the chickpea isolates, maximum P-solubilization was observed with 8C (247 µg/ml) while 13C showed the least (22.7 µg/ml) with a pH decline of 5.45. Among the 68 mustard isolates, maximum P-solubilization was observed with 22M (227.0 µg/ml) followed by 71M (220 µg/ml) and 27M (201 µg/ml), while lowest P-solubilization was shown by 3M (12.6 µg/ml) with a pH of 6.1. Highest P-solubilization was shown by 44W (124 µg/ml) followed by 19W (122 µg/ml), 54W (118 µg/ml) and 51W (116.2 µg/ml) among the 49 isolates from wheat, while lowest was by 1W (2.2 µg/ml) with a pH of 6.7.

The P-solubilization by various isolates on the solid medium and the broth did not show much correlation, the results being in conformity with those reported [7] earlier also. Most of the isolates showed decrease in pH of the medium with large variations, which was due to the release of organic acids by PSB [24, 25]. However, no correlation between reduction in pH and P-solubilization was noticed as the quality and quantity of acids produced differs in different organisms [26, 27]. Aliphatic acids like citrate and fumarate have been reported to be better than aromatic acids in releasing P [28]. Also, di and tribasic acids are more effective than their monobasic versions.

Characterization of PSB isolates: All the 193 PSB isolated from the rhizospheric soil of the three crops were characterized morphologically as well as on the basis of their biochemical analysis, P-solubilization efficiency and intrinsic antibiotic resistance pattern.

1. On the basis of morphological characteristics: Based upon the variations in colony morphology, all the PSB isolates were categorized into 20 groups (Table 2). Among the PSB from chickpea rhizosphere, 17 different types of col-

Table 2 Grouping of PSB isolates from rhizospheres of chickpea, mustard and wheat based on morphological characteristics

Group	Colony characteristics of PSB isolates	Number of isolates			Total isolates (193)
		Chickpea (76)	Mustard (68)	Wheat (49)	
1.	White/off-white, small, round, dry	2	2	1	5
2.	White/off-white, small, round, semi-dry	1	6	4	11
3.	White/off-white, small, round, gummy	2	3	–	5
4.	White/off-white, small, irregular, dry	5	1	–	6
5.	White/off-white, small, irregular, semi-dry	–	2	–	2
6.	White/off-white, small, irregular, gummy	1	–	–	1
7.	White/off-white, large/medium, round, dry	7	7	9	23
8.	White/off-white, large/medium, round, semi-dry	18	20	9	47
9.	White/off-white, large/medium, round, gummy	6	12	3	21
10.	White/off-white, large/medium, irregular, dry	12	2	–	14
11.	White/off-white, large/medium, irregular, semi-dry	7	5	–	12
12.	White/off-white, large/medium, irregular, gummy	6	1	1	8
13.	Pigmented, small, round, dry	1	1	1	3
14.	Pigmented, small, round, semi-dry	–	–	4	4
15.	Pigmented, large/medium, round, dry	1	–	5	6
16.	Pigmented, large/medium, round, semi-dry	–	2	6	8
17.	Pigmented, large/medium, round, gummy	2	4	–	6
18.	Pigmented, large/medium, irregular, dry	2	–	1	3
19.	Pigmented, large/medium, irregular, semi-dry	1	–	3	4
20.	Pigmented, large/medium, irregular, gummy	2	–	2	4

ony morphologies were seen, while the PSB from mustard and wheat rhizosphere showed 14 and 13 different types of colonies. The colony morphology of PSB isolates collected from different locations showed variations with respect to most of the colony characteristics (color, size, shape and texture). Most of the PSB isolates were white/off-white, but a few showed yellow, orange or brown color pigmentation. The size of the colonies varied from very small to large and the texture of the colonies was dry, semi-dry or gummy. Variations in other characters were also observed. Eighteen PSB from chickpea, 20 from mustard and nine from the wheat rhizosphere showing white/off-white and large/medium-sized colonies with a round shape and semi-dried texture constituted the largest group. Gram staining of isolates revealed a fairly homogeneous population of gram-negative, small rods in the rhizosphere of all crops. The PSB diversity based on colony characteristics was higher in chickpea as compared to wheat and mustard as a larger number of samples in chickpea showed 2–3 different types of colonies. The PSB from mustard rhizosphere grown at different locations of Haryana showed least diversity as most of the samples consisted of only a single type of colony morphology. Most of the PSB from wheat samples also showed similar type of colonies; however variations were recorded from location to location.

2. On the basis of biochemical analysis: All the PSB isolates were characterized biochemically by carrying out 20 biochemical tests. Each biochemical character was scored as 1 for a positive reaction and 0 for a negative reaction and the data was analyzed for creating a dendrogram using UP-GMA of SSPC software programme. At a similarity level of 85%, all the PSB strains were divided into nine major groups (Table 3).The first group consisted of 90 (46.6%) isolates (30 from each crop) which was further divided into four subgroups. The second group contained 14 isolates (13 from chickpea and one from wheat) which was further di-

vided into two distinct subgroups, whereas the third group consisted of four chickpea isolates alone. Fourth group had nine isolates (wheat-3, mustard-4 and chickpea-2). Fifth group contained four isolates (wheat-3 and mustard-1) in which the wheat isolate fell in one subgroup while the mustard isolate in another subgroup. Sixth group contained 23 isolates (mustard-14, wheat-2 and chickpea-7) with two subgroups. Seventh group contained four isolates and the eighth group comprised of 41 isolates (19 from chickpea, 13 from mustard and 9 from wheat). Ninth group had two subgroups consisting of a total of four isolates.

Numerical analysis showed that the PSB isolates from chickpea, mustard and wheat rhizosphere from different locations were randomly distributed in all the groups. None of the isolates from different groups were associated with a specific host suggesting a predominantly homogeneous population among these PSB isolated from different crops. P-solubilizing microorganisms constitute 20–40% of the culturable population of soil microorganisms and form a heterologous group dispersed into several genera. Except for phosphate solubilization which is common in these bacteria, these isolates may differ in other biochemical characteristics. This may be the reason that the PSB isolates from a particular host did not fall in one group on the basis of biochemical characterization.

3. On the basis of P-solubilization on solid and in liquid PVK medium: The PSB were also grouped on the basis of PSE and P-solubilization to correlate the P-solubilization with crop and the region of isolation (Table 3). All the 193 isolates were categorized into five groups on the basis of PSE. Forty seven isolates from chickpea showed PSE < 20% while 22 isolates exhibited PSE in the range of 20–50%. Five isolates showed PSE between 50–100% and two exhibited PSE >100%. Fifty-three isolates of mustard showed PSE < 20% and 15 between 21–50%. None of the mustard isolates showed PSE > 50%. Among the wheat

Table 3 Grouping of PSB isolates from rhizosphere of different crops on the basis of biochemical tests

Biochemical group	Number of isolates			Percentage of total isolates in each group
	Chickpea	Mustard	Wheat	(%)
1.	30	30	30	46.67
2.	13	–	1	7.25
3.	4	–	–	2.07
4.	2	4	3	4.66
5.	–	1	3	2.07
6.	7	14	2	11.50
7.	–	3	1	2.07
8.	19	13	9	21.2
9.	1	3	–	2.07
Total	76	68	49	

isolates, 23 showed PSE < 20% while 24 isolates showed PSE in the range of 21–50%, and only two isolates showed PSE between 51–100%. On the whole, maximum number of isolates (69) showed PSE in the range of 0-10%, whereas isolates showing PSE between 11–20% and 21–50% were 54 and 61, respectively. The fourth (51–100%) group consisted of chickpea and wheat PSB isolates only, while the fifth group (> 100%) comprised of chickpea isolates alone. All the mustard isolates showed PSE < 50% and fell in the rest of the three groups.

On the basis of P-solubilization in PVK broth, the PSB were again categorized into five major groups (Table 4). Forty one per cent isolates fell into the first group showing P-solubilization < 50μg/ml, whereas the second group (51–100 μg/ml) and the third group (101–150 μg/ml) consisted of 25 and 21 per cent, respectively. Fourth group included seven per cent PSB isolates showing P-solubilization of 151–200 μg/ml. The fifth group showing maximum P-solubilization (>200 μg/ml) contained only 10 isolates and consisted of about five per cent only. Thirty five isolates of chickpea showed P-solubilization < 100 μg/ml, while 27 isolates showed between 101–150 μg/ml. PSE of 14 isolates ranged between 151–250 μg/ml. Among the 68 mustard isolates, P-solubilization of < 50 μg/ml was noticed with 29 isolates, whereas 30 isolates showed between 51–150 μg/ml and 9 isolates between 151–250 μg/ml. Thirty five isolates from wheat showed P-solubilization < 50 μg/ml, whereas six isolates showed solubilization in the range of 51–100 μg/ml and eight between 101–150 μg/ml. None of the isolates showed P-solubilization >150 μg/ml. This large variation in P-solubilization of the isolates can be ascribed to the variations in their metabolic activity as also reported earlier [29, 30]. However, P-solubilization by different isolates was not influenced by the host plant and location.

4. On the basis of the intrinsic antibiotic resistance pattern: Intrinsic antibiotic resistance (IAR) 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 IAR pattern was used as a criterion to know the diversity among the 193 isolates using eight antibiotics. All the 193 isolates (Table 5), irrespective of the host plant, were resistant to streptomycin (99%) and ampicillin (100%), and a great majority were also resistant to penicillin (96%). Fifty percent isolates from chickpea, mustard and wheat were resistant to Cm. The isolates were comparatively less resistant to kanamycin (35%), neomycin (46%) and tetracycline (37%); however, chickpea and wheat isolates showed higher resistance than the mustard isolates to these antibiotics. All the PSB isolates showed least resistance to nalidixic acid which inhibited 92, 99 and 72% of the isolates from chickpea, mustard and wheat, respectively.

Based on the resistance to the number of antibiotics, the PSB isolates were categorized into seven groups (Table 6). Only 17 isolates from chickpea (6), mustard (1) and wheat (10) showed resistance to all the eight antibiotics tested. Maximum number of the isolates belonged to the sixth group showing resistance to Sm, Am and Pn. Fourteen isolates belonged to the 7th group consisting of isolates resistant to either Sm-Am or Am-Pn and rest of the categories contained 20–30 isolates. This shows that the the PSB isolates were mostly resistant to Sm, Am, and Pn. The results show that the IAR of different isolates is not confined to any particular crop or region of Haryana. Diversity in soil bacteria using IAR pattern has been demonstrated in earlier studies [31, 32] also.

The results in the present study show that a large and diverse repository of PSB exists in the rhizosphere of chick-

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

Group of isolates		Chickpea (76)	Mustard (68)	Wheat (49)	Total (193)
P-solubilization efficiency (PSE-%)					
I	< 10	23 (30)	30 (44)	16 (33)	69 (36)
II	11–20	24 (32)	23 (34)	7 (14)	54 (30)
III	21–50	22 (30)	15 (22)	24 (49)	61 (32)
IV	51–100	05 (07)	–	02 (04)	07 (04)
V	> 100	02 (03)	–	–	02 (01)
P-solubilization (μg/ml)					
I	< 50	16 (21)	29 (43)	35 (71)	80 (41)
II	51–100	19 (25)	24 (35)	06 (12)	49 (25)
III	101–150	27 (36)	06 (09)	08 (16)	41 (21)
IV	151–200	07 (09)	06 (09)	–	13 (07)
V	201–250	07 (09)	03 (04)	–	10 (05)

Values in parentheses represent percentage of the total isolates of the respective crops

Table 5 Intrinsic antibiotic resistance of PSB isolates from rhizosphere of different crops grown in different regions of Haryana

Antibiotic	Concentration (µg/ml)	Chickpea (76)	Mustard (68)	Wheat (49)	Total (193)
		Number of isolates resistant to different antibiotics			
Kanamycin (Km)	100	33 (43)	12 (18)	22 (45)	67 (35)
Neomycin (Nm)	100	45 (59)	23 (34)	21 (43)	89 (46)
Streptomycin (Sm)	100	74 (97)	68 (100)	49 (100)	191 (99)
Ampicillin (Am)	100	76 (100)	68 (100)	49 (100)	193 (100)
Nalidixic acid (Nx)	100	06 (08)	01 (01)	14 (29)	21 (11)
Chloramphenicol (Cm)	50	38 (50)	34 (50)	26 (53)	98 (51)
Tetracyclin (Tc)	10	43 (57)	07 (10)	21 (43)	71 (37)
Penicillin (Pn)	100	71 (93)	63 (93)	42 (86)	186 (96)

Values in parentheses represent percentage of isolates resistant to antibiotics

Table 6 Grouping of PSB isolates from different crops based on the number of antibiotics to which they are resistant

Group	Antibiotics	Number of isolates resistant			Total
		Chickpea	Mustard	Wheat	
1.	Km Nm Sm Am Nx Cm Tc Pn	6	1	10	17
2.	Km Nm Sm Am Cm Tc Pn	18	–	8	26
3.	Km Nm Sm Am Cm Tc	1	1	–	2
	Km Nm Sm Am Cm Pn	–	9	–	9
	Km Nm Sm Am Tc Pn	5	–	1	6
	Nm Sm Am Tc Pn Cm	2	2	–	4
4.	Km Nm Sm Am Pn	2	–	–	2
	Km Sm Am Nx Pn	–	–	3	3
	Km Sm Am Cm Pn	–	1	–	1
	Nm Sm Am Cm Pn	2	8	1	11
	Nm Sm Am Tc Pn	3	–	–	3
	Sm Am Cm Tc Pn	5	3	1	9
5.	Km Sm Am Pn	1	–	–	1
	Nm Sm Am Pn	6	2	–	8
	Sm Am Cm Pn	4	9	5	18
	Sm Am Tc Pn	4	–	–	4
6.	Sm Am Nx	–	–	1	1
	Sm Am Cm	–	1	–	1
	Sm Am Tc	–	–	1	1
	Sm Am Pn	10	29	12	51
7.	Sm Am	5	2	5	12
	Am Pn	2	–	–	2
	Total isolates	76	68	49	193

pea, mustard and wheat crops in the Haryana state. A greater number and a higher diversity was observed in the isolates of chickpea rhizosphere as compared to the isolates of mustard and wheat. Although the PSB were widely distributed throughout the state and variations were observed within the isolates of each crop but no significant differences were observed in the PSB count from the rhizospheric soil of the south-west and the north-east zone of Haryana. The total number of PSB was almost uniform throughout the state with only a few exceptions. However, characterization of the isolated PSB on the basis of morphological characteristics, biochemical analysis and on P-solubilization revealed that the isolates were highly diverse with respect to their size, color, shape, texture, and in their ability to solubilize

phosphorous and to react biochemically. The PSB diversity was noticed highest in chickpea, followed by wheat and least by mustard isolates for all the parameters studied. Collection of these diverse types of PSB can help in the selection of highly efficient strains having the possibility to be used as bioinoculants for crop productivity.

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