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Research Article

Isolation and Characterization of Phosphate Solubilizing Bacteria from Cultivated Fields of South and North Gondar Zones of Western Ethiopia

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Abstract

Chemical fertilizers are used broadly in modern agriculture to improve the crop productivity throughout the world to meet the growing demand for food. Some of these chemical fertilizers are Phosphorus (P) fertilizers used to improve crop yields. P is required by the plant for its growth next to nitrogen. Biofertilizers are used to replace these chemical fertilizers to some extent among in which Phosphate-solubilizing bacteria (PSB) are gaining attention. In the current study, phosphate-solubilizing bacterial strains were isolated from soils collected from cultivated fields of different localities in South and North Gondar zones. Forty six PSB strains were isolated out of which nine efficient PSB isolates were selected for morphological and biochemical characterization based on their growth and phosphate solubilizing ability on Pikovskaya's agar medium. The isolated PSB strains showed a clearing zone ranging from 6 mm to 12 mm in diameter, where WP1 and WP2 showed maximum solubilization of 12 mm and 10 mm respectively. Most of the bacteria isolated were gram negative rods, few were gram positive and the biochemical tests revealed that these strains belong to *Pseudomonas* sp. and *Bacillus* sp. respectively.

Keywords: Phosphate solubilization, biofertilizers, rhizosphere, chickpea, maize.

Introduction

Chemical fertilizers are used broadly in modern agriculture to improve the crop productivity throughout the world to meet the growing demand for food. These chemical fertilizers like NPK no doubt are improving crop productivity at the same time they are hazardous to the environment. There is a need to replace these synthetic fertilizers with eco-friendly biofertilizers. Bioinoculants/biofertilizers are gaining importance in modern agriculture which includes beneficial live microbial formulations of nitrogen fixing bacteria like *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., cyanobacteria, Potassium solubilizing bacteria, plant growth promoting bacteria, phosphate solubilizing bacteria and vesicular arbuscular mycorrhiza (VAM). They are gaining importance because of ecofriendly, non-hazardous, and nontoxic nature (Kannaiyan *et al*., 2004). Phosphorus (P) is second only to nitrogen as most essential macro-nutrient required by plants (Azziz *et al*., 2012). Organic matter derived from dead and decaying plant debris is rich in organic sources of phosphorus. Though adequate amount of P is available in the soil, plants experience its deficiency partly may be due to the reason that plants are capable of absorbing P only in available form. The P may undergo fixation with other metallic elements present in rhizosphere zone which makes it insoluble (Gaind and Gaur, 1999). An enormous proportion of soluble P is precipitated rapidly into insoluble forms, particularly Fe-P and Al-P complexes, which can be unavailable to plants (Gyaneshwar *et al*., 2002). The phosphorus deficiency of the soil can be corrected by inoculating seeds with PSB along with phosphatic fertilizers (Rafi, 2007). Phosphate-solubilizing microorganisms which include both bacteria (PSB) and VAM fungi are important in solubilizing insoluble phosphate to available form. Solubilization of insoluble P by microorganisms was reported by Pikovskaya, (1948). There are reports on different mechanisms for phosphate solubilization. The principal mechanisms include, production of organic acids (Maliha *et al.*, 2004; Hu *et al.*, 2009); production of acid phosphatase (Turner and Haygarth, 2005). Several workers isolated PSB from various soils from different regions of the world (Sharma *et al.*, 2011; Gupta *et al.*, 2012; Ranjan *et al*., 2013; Baliah *et al*., 2016; Paul and Sinha, 2017). In the present study efforts were made to isolate indigenous phosphate solubilizing bacteria from different locations of Gondar, Ethiopia.

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Table 1. Morphological Characters of PSB isolates.

PSB isolate/ Colony character	WP1	WP2	WP4	WP6	TP1	FP4	GZP2	FP2	GZP4
Shape	irregular	irregular	irregular	irregular	irregular	irregular	irregular	irregular	irregular
Margins	undulate	undulate	undulate	undulate	undulate	entire	undulate	entire	undulate
Elevation	raised	raised	raised	raised	raised	flat	raised	raised	flat
Size	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Texture	smooth	smooth	smooth	smooth	smooth	rough	smooth	smooth	rough
Appearance	dull	dull	glistening	glistening	glistening	dull	glistening	dull	glistening
Pigmentation	white	white	white	cream	yellow	cream	white	yellow	cream
Optical property	opaque	opaque	opaque	opaque	opaque	opaque	opaque	opaque	opaque
Gram staining reaction	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
Cell shape	rod	rod	rod	rod	rod	rod	rod	rod	rod
Motility	+	+	+	+	+	+	+	+	+
Halo zone diameter (mm)	12	10	8	9	8	7	8	6	5

Materials and methods

Collection of soil samples: Soil samples were collected from the maize, chickpea and fababean cultivated fields of different locations Fogera, Farta, Libokemkem of South Gondar zone and Wogera, Dembia, Debark, Gondar Zuria, Gondar Town administrative districts of North Gondar zone, Western Ethiopia. The soils were collected from a depth of 15 cm at random in the fields and mixed well to represent a composite soil sample, placed in sterilized plastic bags and were transported to the microbiology laboratory of College of Agriculture and Rural Transformation for isolation of Phosphate Solubilizing Bacteria (PSB).

Isolation of Phosphate Solubilizing Bacteria (PSB): The soil samples were subjected to serial dilution in phosphate buffer saline (pH 7.2) and were plated on Pikovskaya's agar medium (PVK) plates of the following composition, g/L (modified by Sundara Rao and Sinha (1963): Glucose -10.00; $Ca_3(PO_4)_2$ -5.0; $(NH_4)_2SO_4$ -0.50; KCl -0.20; $MgSO_4.7H_2O$ -0.10; $MnSO_4.H_2O$ -Traces; $FeSO_4.7H_2O$ -Traces; Yeast extract -0.50; Agar agar-20.00. pH was maintained at 7. Here tricalcium phosphate (TCP) is the sole phosphate source. The inoculated plates were incubated at 30±2°C for 7 d and observed for halo zone formation. The colonies forming clear halo zones around them indicating phosphate solubilization were picked up by an inoculation loop and streaked on fresh Pikovskaya's agar plates, incubated as described above (De Freitas *et al.*, 1997). This procedure was repeated 4-5 times until isolated, discrete, morphologically uniform colonies were obtained. The strains which showed clear zones of phosphate solubilization were transferred on to fresh Pikovskaya's agar slants. The slants were then incubated at 30±2°C until a good growth was obtained and preserved at 4°C for further use.

Morphological, biochemical characterization of the isolates: Morphological characters such as colony colour, size, shape, elevation, surface texture, margins, and consistency were observed for their characterization.

Gram staining: The isolated PSB strains were subjected to Gram staining using the standard procedure (Anonymous, 1957). The stained cells were observed under compound microscope for their Gram reaction. The isolated PSB strains were also tested for catalase activity (Graham and Parker, 1964), oxidase (Kovaks, 1956), starch hydrolysis and other biochemical tests (Blazevic and Ederer, 1975).

Results and discussion

Isolation of PSB: Phosphate solubilizing bacteria are concentrated highly in the rhizosphere region and are metabolically active. In the present study, the collected soil samples were plated on Pikovskaya's (PVK) agar plates for PSB isolation. The bacterial strains forming clear phosphate solubilization zone (PSZ) after incubation were selected and observed for colony characteristics. Forty six bacterial strains were isolated from different soil samples collected and were repeatedly streaked on PVK agar plates to confirm their P-solubilizing efficiency. By observing the growth PVK medium, nine PSB strains were found to be effective P-solubilizers based on the diameter of the PSZ around the colonies. The isolates showed PSZ ranging from 6 mm to 12 mm diameter after 7-8 d of incubation at 32°C.

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Table 2. Biochemical characteristics of PSB isolates.

PSB Isolate/ Characteristics	WP1	WP2	WP4	WP6	TP1	FP4	GZP2	FB2	GZP4
Growth at 5% NaCl	+	+	+	+	+	+	+	+	+
Growth at 4°C	-	-	-	-	-	-	-	-	-
Growth at 37°C	+	+	+	+	+	+	+	+	+
Growth at 42°C	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+
Indole production test	-	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-	-
VogeseProskauer test	-	-	-	-	-	-	-	-	-
Citrate utilization test	+	+	+	+	+	-	+	-	+
Urease activity	-	-	-	-	-	-	-	-	-
Gelatine liquefaction	+	+	+	+	+	+	+	+	+
Hydrolysis of starch	+	-	-	-	+	-	-	-	-
Hydrolysis of casein	+	+	+	+	-	-	+	-	+
Utilization of sugars									
Glucose	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-
Fructose	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+
Maltose	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-

The bacterial strains WP1 (12 mm) and WP2 (10 mm) showed maximum and strains FP2 (6 mm) and GZP4 (7 mm) showed minimum phosphate solubilization. The development of halo zone around the bacterial colonies might be due to the production of low molecular weight organic acids by phosphate solubilizing bacterial strains (Halder and Chakrabartty, 1993; Goenadi *et al*., 2000; Rashid *et al.,* 2004; Sharma, 2005; Paul and Sinha, 2013). For solubilization of insoluble phosphates different organic acids such as gluconic acid, 2-ketogluconic acid, lactic acid, isovaleric acid, isobutyric acid, acetic acid, oxalic acid, citric acid are produced by PSM (Rodriguez and Fraga, 1999). These organic acids can either dissolve phosphates as a result of anion exchange or can chelate Ca, Fe or Al ions associated with the phosphates (Gyaneshwar *et al*., 2002). However, soil bacteria vary significantly in their ability to secrete organic acids and thereby, solubilize mineral phosphates at different extent.

Colony characteristics: All the bacterial isolates produced typical colonies which showed variable morphological characters. Most of the isolates formed well developed colonies on PVK agar medium which were white, cream and yellow in color, some raised, some were flat with irregular margins. Most of the isolates were found to be rod shaped and gram negative, some were gram positive rods.

Morphological characters of the selected PSB isolates are represented in Table 1 and biochemical tests of different types were performed to characterize the PSB isolates as shown in Table 2. Out of nine isolates, seven showed positive tests which are reliable with the characteristic features of the genus *Pseudomonas* and two of the isolates resembled the characters of genus *Bacillus*. The results were in agreement with the findings of several workers as reported elsewhere that *Pseudomonas* sp. were known for their phosphate solubilization (Hameeda *et al.,* 2008; Ahemad and Khan, 2011; Paul and Sinha, 2013; Ranjan *et al*., 2013; Oteino *et al*., 2015). *Bacillus* sp. were also were established to be as efficient phosphate solubilizers (Rojas *et al*., 2001; Egamberdiyeva, 2005; Guneş *et al.,* 2009; Baliah *et al*., 2016; Rafique *et al*., 2017). Behera *et al.* (2017) isolated a phosphate solubilizing bacterium, PSB-37, from mangrove soil and was identified as *Serratia* sp.

Conclusion

In the present study, a total of nine phosphate solubilizing bacteria were found to be efficient in phosphate solubilization isolated from the maize, chickpea and fababean cultivated soils. These were tentatively identified as *Pseudomonas* sp. and *Bacillus* sp. by observing morphological, cultural and biochemical tests. The PSB strains WP1 and WP2 showed maximum and strains FP2 and GZP4 showed minimum phosphate solubilization.

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Further studies at molecular level are needed for the identification of PSB strains up to the species level.

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