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Research Article

ISOLATION AND CHARACTERIZATION OF INDUSTRIALLY IMPORTANT BIOCATALYSTS-PRODUCING BACTERIA FROM WASTE DISPOSAL LAND

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ABSTRACT
Modern biology has progressively increasing demand for novel biocatalysts. Lipases, amylases, proteases, cellulase etc. are among the most important biocatalysts due to their enormous industrial applications. Therefore, screening of these industrially important biocatalyst-producing microorganisms from microbial diverse ecosystem, such as soil could facilitate the finding of novel industrially important enzymes including lipases, amylases, proteases etc. In the present study, among the 91 soil isolates from waste disposal land, Kolkata, India, 13 bacterial isolates were selected for further studies based on their ability to produce extracellular industrially important enzymes as well as plant growth promoting metabolites. The 76.9, 100, 30.7 and 100% of the isolates tested here were positive for the production of lipase, protease, amylase and cellulase, respectively. All of these 13 isolates tested here were able to produce plant growth-promoting substances such as indole acetic acid (IAA) and also could solubilize inorganic phosphate. However, none of them were positive for the production of hydrogen cyanide and chitinase activity.

Keywords: *Industrially Important enzymes, Bacterial Lipase, Amylase, Protease, Phosphate Solubilization, Indole Acetic Acid*

INTRODUCTION
To understand the biochemical processes of life, it is often easier to study them in a simple system like microorganisms as they have many of the same properties as more complex organisms. They also contain some unique properties such as production of novel enzymes, ability to degrade waste products etc. Therefore, many scientific approaches have been developed to study the biodiversity as well as novel enzymes of a particular environmental sample such as soil or marine water. Enzymes are considered nature's catalysts. These biocatalysts include lipases, amylases and proteases and these are currently attracting enormous attention because of their biotechnological potential. However, the enzymes belonging to a limited species have been established to have ample stability and biosynthetic capabilities to allow custom use in industries/ biotechnological purposes. Therefore, search for a wider range of these enzymes types would be advantageous for the application in detergent, food processing, textile as well as pharmaceutical industries.

Lipases are triaclyglycerol acylhydrolases (EC 3.1.1.3) that can hydrolyse fats into fatty acids and glycerol at aqueous condition and can reverse the reaction in non-aqueous media. Lipases are of special interest in industrial applications due to their high stability towards extreme temperatures, alkaline pH and in presence of organic solvents and chemo, regio and enantioselectivity (Saxena *et al*., 2003; Ghosh *et al*., 1996). Among all lipases, microbial lipases are immensely useful in industrial applications, as microbes which produce a wide variety of extracellular lipase (Akimoto *et al*., 1999), can easily be cultivated and can catalyse a large variety of hydrolytic and synthetic reactions (Godfrey *et al*., 1996). Lipolytic microbes are largely found in diverse habitats such as domestic and industrial waste disposal land, oil contaminated soil, vegetable oil processing factories, decaying food, dairy industries, compost heaps, coal tips and hot springs (Wang *et al*., 1995). Lipases are also widely used in biopolymer synthesis, biodiesel production, and treatment of fat containing waste effluents.

Amylase can be obtained from several fungi, yeast, bacteria and actinomycetes; however, enzyme from fungal and bacterial sources has dominated applications in industrial sectors. The application of an amylase in industrial reactions depends on its unique characteristics, such as its action pattern, substrate specificity, major reaction products, optimal temperature, and optimal pH. Amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile, paper, pharmaceutical to sugar industries (Kunamneni *et al*, 2005). Conversion of starch into sugar, syrups and dextrins by amylase enzyme forms the major part of starch processing industry (Aiyer, 2005). Microbes are good source for proteases. The use of alkaline protease as an active ingredient in laundry detergents is well known (Sinha and Satyanarayana, 1991). Alkaline proteases are also widely used in leather processing (Aunstrup, 1983), medical diagnostics, recovery of silver from X-ray films, meat, photographic and dairy industries (Kalisz, 1988).

Modern biology has a steadily increasing demand for novel biocatalysts. Lipases, amylases, cellulases and proteases are among the most important biocatalysts due to their huge industrial applications. Although lipases are produced by plants, animals and microorganisms, microbial lipases have an immense prospective for commercial applications due to their stability, selectivity and broad substrate specificity. Among industrially important enzymes from bacteria, attention has usually been focused on particular classes of enzymes such as the lipases from the genus *Pseudomonas*, which are especially interesting for biotechnology, or esterases possibly involved in bacterial pathogenicity. Unfortunately, information on the relatedness of the numerous bacterial industrially important/ biotechnological potential enzymes studied so far is incomplete and scattered in the literature. The biochemical and genetic aspects of these enzymes from fungi have been widely studied. Usefulness of bacterial enzymes, mostly those produced by a variety of species of a limited genus in the industry/ biotechnology has been demonstrated previously. Therefore, screening of these industrially important biocatalyst-producing microorganisms from microbial diverse ecosystem, such as soil could facilitate the finding of novel industrially important enzymes including lipase, amylase, cellulase and protease. Therefore the objective of this study was the isolation and characterization of microbes from waste disposal land with particular reference to their ability to produce industrially important enzymes such as lipase, amylase, cellulase and protease.

MATERIALS AND METHODS

Sample Collection

Soil samples are collected from different places of Dhapa field, a waste disposal land of Kolkata Municipality Corporation, situated near E. M. Bypass, West Bengal, India.

Isolation of bacterial strains from soil samples

To isolate cellulase-producing bacteria, soil samples were collected from 50 different places of waste disposal land, Kolkata, 1 gm. of soil sample was suspended in 10ml of 10 mM phosphate buffered saline (PBS; pH 7.2) and kept at room temperature for 30 min. Supernatant was collected and 10^{-1} dilution was made. From this dilution, 100 µl was plated on CMC agar media [Carboxymethyl cellulose (CMC) 1.0 %, sodium nitrate ($NaNO_3$) 0.65%, potassium chloride (KCl) 0.65%, K_2HPO_4 0.65%, yeast extract 0.03%, magnesium sulphate ($MgSO_4.7H_2O$) 0.3%, dextrose 0.065% and agar powder 2%; pH 7.2] and incubated for 48 h at 37°C. Zone of clearance/yellowish surrounding the colony upon flooding of the plates with Congo red solution (1%) and destaining with 0.1 M NaCl against dark red background indicates cellulase positive bacteria. These strains were stored both as nutrient agar stab culture in room temperature and in 20% glycerol stock at -80°C.

Morphological and Biochemical characterization of soil isolates

A series of biochemical tests were performed to characterize the isolated strains; such as, Gram staining, determination of shape and motility of the isolates, acetoin production, methyl red test, indole production test, gelatin hydrolysis, urease test, oxidase test, nitrate reductase assay, Simmons citrate test, sugar fermentation test and amino acid utilization test etc. (Collee *et al*., 1996). The shape and motility were determined by phase contrast microscope (Olympus BX51/B52).

Lipase assay

Isolated strains were spotted on lipase selection agar media (NaCl 0.5%, yeast extract 0.5%, Tryptone 1.0%, and agar powder 1.8%; pH 7.2) containing 1% (v/v) tributyrin and 0.25% (v/v) Tween 80. The plates were incubated at 37°C for 24 hours. Formation of a clear zone surrounding the colony indicates lipolytic bacterial strains.

Lipase was routinely assayed spectrophotometrically (Jasco V630, USA) using p–nitro phenyl palmitate (*p*–NPP) (Sigma) as substrate (Salameh and Wiegel, 2007). The reaction mixture contained 405 µl of freshly prepared buffer (10 mg of gum arabic in 10 ml of 50 mM Tris–HCl buffer; pH 8.0) and 45 µl of substrate (*p*-NPP; final concentration in the assay, 0.27mg ml^{-1}) and this mixture was emulsified for 2 min in an ultrasonic bath. This freshly prepared substrate solution was pre warmed at 37°C and then mixed with 50 µl of enzyme solution (cell-free culture supernatant). After 5 minutes of incubation at 37°C, the reaction was stopped by the addition of 0.5 ml of chilled 0.25M sodium carbonate. The reaction mixture was cleared by centrifugation before the amount of liberated *p*-nitro phenol was determined spectrophotometrically (Jasco V630, USA) at A_{410}. One enzyme unit is defined as 1 nmol of *p*-nitro phenol enzymatically released from the substrate ml^{-1} min^{-1} (Salameh and Wiegel; 2007).

Amylase assay

Isolated strains were spotted on starch agar media (Peptone 0.5%, beef extract 0.3%, NaCl 0.5%, soluble starch 0.5% and agar powder 2%; pH 7.2) and incubated for 48 hours at 37°C. Zone of clearance surrounding the colony upon flooding of the plates with iodine/potassium iodide solution against dark blue background indicates amylase positive bacteria.

For quantitative amylase assay, amylase-positive bacteria were grown in amylase selection media (Peptone-0.5%, beef extract-0.3%, NaCl-0.5%, soluble starch-0.5%; pH 7.2) for 24 h at 37°C under shaking condition. After incubation, cells were centrifuged at 10000 rpm for 10 min at 4°C. Clear supernatant were then used as crude amylase. To measure amylase activity, 0.5 ml of clear cell extract was added to 1 ml of reaction buffer composed of 0.1 M phosphate buffer at pH 7.0 containing 0.5 ml of 0.5% (w/v) soluble starch. Reactions were carried out at 37°C for 10 minutes. 2 ml of dinitrosalicyclic acid solution was then added and boiled the sample for 5 min in boiling water bath. After cooling down the solution at room temperature, 10 ml of sterile milliQ water was added and the O.D. was measured at 540 nm (Jasco V630, USA). The released reducing sugars were measured as D-glucose equivalents (Miller *et al*., 1959). One unit (U) of amylase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar per minute.

Protease assay

Isolated strains were spotted on protease selection agar media (Peptone 0.5%, beef extract 0.3%, NaCl 0.5%, skimmed milk 1.5% and agar powder 2%; pH 7.2) and incubated for 48 h at 37°C. Zone of clearance surrounding the colony indicates protease positive bacteria.

For quantitative estimation, bacterial strains were grown in protease selection media (Peptone-0.5%, beef extract-0.3%, NaCl-0.5%, skimmed milk-1.5%, pH 7.2) for 24 h at 37°C under shaking condition. After incubation cells were centrifuged at 10,000 rpm for 10 min at 4°C. Clear supernatant were then used as crude protease (Anson, 1938). Protease activity was assayed following the previously described method (Folin and Ciocalteau, 1927; Lowry *et al*., 1951). 5 ml of 2% skimmed milk solution was digested with 1.0 ml of culture supernatant dissolved in 0.1 M Tris-HCl buffer; pH 8.0 at 37°C for 10 min. The enzyme reaction was terminated by the addition of 5 ml of trichloroacetic acid solution (0.11 M) and the mixture was filtered. Two ml of the filtrate was taken in a test tube, and 5 ml of 0.44 M sodium carbonate and 1 ml of the 0.5 N Folin-Ciocalteau reagent were added while the solution was kept at 37°C during addition of the phenol reagent. After standing for 20 minutes, the colour was read at 660 nm in a spectrophotometer (Jasco V630, USA) against a standard (10^{-4} M tyrosine) prepared in the same manner (Thangam and Rajkumar, 2002).

Cellulase assay

Isolated strains were spotted on CMC agar media [Carboxymethyl cellulose (CMC) 1.0 %, sodium nitrate ($NaNO_3$) 0.65%, potassium chloride (KCl) 0.65%, K_2HPO_4 0.65%, yeast extract 0.03%, magnesium sulphate ($MgSO_4.7H_2O$) 0.3%, dextrose 0.065% and agar powder 2%; pH 7.2] and incubated for 48 h at 37°C. Zone of clearance/yellowish surrounding the colony upon flooding of the plates with Congo red solution (1%) and destaining with 0.1 M NaCl against dark red background indicates cellulase positive bacteria.

For quantitative estimation, CMCase positive bacteria were grown in CMCase selection media (CMC-1.0 %, $NaNO_3$-0.65%, KCl- 0.65%, K_2HPO_4-0.65%, yeast extract-0.03%, $MgSO_4.7\ H_2O$- 0.3%, dextrose-0.065%; pH 7.2) for 24 hours at 37°C in shaking condition. After incubation, cells were centrifuged at 10000 rpm for 10 min at 4°C. Clear supernatant were then used as crude CMCase. To measure CMCase activity, 0.5 ml of clear cell extract was added to 1 ml of reaction buffer composed of 0.1 M phosphate buffer at pH 7.0 containing 0.5 mL of 0.5% (w/v) CMC powder. Reactions were carried out at 37°C for 10 minutes. 2 ml of dinitrosalicyclic acid solution was then added and the sample was kept in boiling water bath for 5 minutes. After cooling down the solution at room temperature, 10 ml of sterile milliQ water was added and the O.D was measured at 540 nm (Jasco V630, USA). The released reducing sugars were measured as D-glucose equivalents described by Miller; 1959. One unit (U) of cellulase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar per minute (Miller; 1959).

Chitinase assay

Isolated strains were spotted on colloidal chitin agar media (Colloidal Chitin 0.5 %, $NaNO_3$ 0.65%, KCl 0.65%, yeast extract 0.03%, $MgSO_4.\ 7\ H_2O$ 0.3%, $MgCl_2.6H_2O$ 0.5%, $(NH_4)_2SO_4$ 0.05%, dextrose 0.1% and agar powder 2%; pH 7.2) and incubated for 72 hours at 37°C. Zone of clearance or yellowish zone surrounding the colony against dark red background indicates chitinase positive bacteria upon flooding of the plates with Congo red solution (1%) and destaining with 0.1 M NaCl (Yuli et al.; 2004).

Hydrogen cyanide (HCN) production assay

Qualitative determination of cyanide was carried out using the method described by Lorck (1948) following the modification by Alstrom and Burns (1989). A media (Peptone 0.5%, beef extract 0.3%, NaCl 0.5%, 4.4g/l glycine and agar 1.5%; pH 7.0) was prepared for cyanide production assay. Briefly, 10 ml of this media in a sealed screw cap tubes with a hanging filter paper strip soaked with colour developing solution (0.5% picric acid and 2% sodium carbonate) was inoculated with an isolate and incubated at 30°C for 72 hours. Development of a change of colour from yellow to orange, red, or brown was recorded as an indication of weak, moderate, or strongly cyanogenic potential (HCN production ability), respectively.

Indole acetic acid (IAA) production assay

5 mL Luria Broth supplemented with 1 mM tryptophan was inoculated with the isolate and incubated at 30°C for 72 hours under shaking condition. Cultures were centrifuged at 10000 rpm for 10 minutes and clear supernatant were used for the estimation of IAA production (Joseph *et al*., 2007). Briefly, 2 ml of supernatant was acidified with 2 drops of ortho phosphoric acid and 4 ml of Salkowski reagent was added (1 ml 0.5 M $FeCl_3$ in 50 ml 35% perchloric acid), mixed thoroughly and incubated at room temperature for 30 minutes for the development of pink colour. Optical density of the mixture was measured at 530 nm and IAA was determined (µg/mL) from the standard curve using pure IAA as standard.

Mineral phosphate solubilization activity

Mineral phosphate solubilization activity was screened by plating the isolates on modified Pikovskaya's agar medium (PKV) (Calcium phosphate, β-$Ca_3\ (PO_4)_2$ 0.25%, KCl 0.02%, $MgSO_4.7H_2O$ 0.025%, magnesium chloride ($MgCl_2.6H_2O$) 0.5%, yeast extract 0.05%, ammonium sulphate $(NH_4)_2SO_4$ 0.05%, bromophenol blue 0.001%, Agar 2%; pH 8.0) and incubated at 30°C for 72 hours. Solubilization of mineral phosphate was detected by observing a clear halo surrounding the bacterial colonies (Gupta et al.; 1994).

For quantitative estimation, the modified PKV broth was inoculated with the isolate and incubated at 30°C for 4 days under shaking condition at 180 rpm. After incubation, the cultures were harvested by centrifugation at 8,000×g for 10 minutes where sterile uninoculated medium was served as control. Remaining phosphate in the culture supernatant was estimated (Fiske and Subbarow, 1925). Cell–free culture supernatant (500 µl) was mixed with 500 µl of 10% (w/v) trichloroacetic acid in a test tube and 4 ml of colour reagent [1:1:1:2 ratio of 3 M H_2SO_4; 2.5% (w/v) ammonium molybdate; 10% (w/v) ascorbic acid and distilled water] was added and incubated at room temperature for 15 minutes. The absorbance of the blue colour developed was measured at 820nm using a spectrophotometer (Jasco V630, USA). The amount of soluble phosphorus was determined from the standard curve of potassium dihydrogen phosphate (KH_2PO_4). All phosphate determinations were made in triplicate.

RESULTS AND DISCUSSION

Among the 91 soil isolates, 13 bacterial strains were selected for further studies based on their ability to produce extracellular industrially important enzymes as well as plant growth promoting metabolites. Among 13 bacterial strains, 4 were Gram-positive and rest 9 were Gram -negative in nature. All the isolates are mesophilic in nature. Biochemical characteristics of these isolates are shown in Table 1.

Table 1: Biochemical characteristics of the bacterial isolates isolated from soil (waste disposal land)

Biochemical Tests	L3	L6	L7	L8	L9	L10	L11	L12	L13	P5	P7	P8	A1
Shape	Rod	Rod	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Cocci	Rod	Rod	Rod
Gram-reaction	+	-	-	-	+	-	+	-	-	+	-	-	-
Motility	+	+	+	-	+	+	+	+	+	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	-	-	+	+	+	+	-	+	+	-	+	+	+
V-P	+	+	+	+	+	+	+	+	+	-	+	+	+
Citrate	+	+	+	-	+	+	+	+	+	+	-	-	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	+	-	-	-	-	-	-	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	-	-	-	+
Fructose	+	+	+	+	+	+	+	+	+	-	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	-	+	+	-	-	+	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine	-	+	+	-	+	+	+	+	-	-	-	-	-
Arginine	-	-	-	+	-	-	-	-	+	-	+	-	+
Ornithine	-	-	-	-	-	-	-	-	-	-	-	-	-

*V-P, Voges-Proskauer**;*** ***(-)*** *= Negative result;* ***(+)*** *= Positive result*

Twelve (92.3%) and 9 (69.2%) of the isolates were positive for Voges-Proskauer, and methyl red tests, respectively. None of them were positive for indole production test. Some (8) of the isolates were motile. Ten (76.9%), 13(100%), 4 (30.7%) and 13 (100%) isolates showed positive results for citrate utilization, oxidase test, nitrate reduction test and hydrolysis of gelatin, respectively. In sugar fermentation test, 13 (100%), 10 (76.9%), 12 (92.3%) and 6 (46%) isolates were positive for dextrose, sucrose, fructose and mannitol,respectively and none of them were positive for arabinose, inositol and sorbitol. For amino acid utilization test, 6 (46%) and 4 (30.7%) isolates were positive for lysine and arginine, respectively and none of them was positive for ornithine utilization.

Table 2: Production of Industrially important extracellular enzymes by the soil isolates

Strains	Lipase (U/mL ± SE) [1]	Protease (U/mL ± SE) [2]	Amylase (U/mL ± SE) [3]	Cellulase (U/mL ± SE) [4]
L3	8.79 ± 0.040	1.010 ± 0.0006	-	0.570 ± 0.0003
L6	2.36 ± 0.010	0.710 ± 0.0003	-	0.556 ± 0.0003
L7	6.62 ± 0.020	1.014 ± 0.0003	-	0.611 ± 0.0003
L8	6.24 ± 0.070	1.418 ± 0.0003	-	0.659 ± 0.0003
L9	1.58 ± 0.001	0.723 ± 0.0003	-	0.605 ± 0.0003
L10	1.69 ± 0.001	0.901 ± 0.0006	-	0.618 ± 0.0003
L11	8.18 ± 0.007	1.545 ± 0.0003	-	0.656 ± 0.0003
L12	1.34 ± 0.010	1.305 ± 0.0003	-	0.772 ± 0.0003
L13	3.93 ± 0.280	1.088 ± 0.0003	0.342 ± 0.0003	0.570 ± 0.0003
P5	-	1.904 ± 0.0003	-	0.643 ± 0.0003
P7	4.52 ± 0.080	3.799 ± 0.0003	0.428 ± 0.0003	0.546 ± 0.0003
P8	-	3.448 ± 0.0003	0.663 ± 0.0003	0.572 ± 0.0003
A1	-	2.308 ± 0.0003	1.424 ± 0.0003	0.723 ± 0.0003

[1]*One enzyme unit is defined as 1µmol of p-nitro phenol enzymatically released per min using p-nitro phenyl palmitate (p-NPP) as substrate.*
[2]*One unit protease activity is defined as 1µmol of tyrosine released per minute using skimmed milk as substrate.*
[3]*One unit amylase activity is defined as 1µmol of D-glucose released per minute using soluble starch as substrate.*
[4]*One unit cellulase activity is defined as 1µmol of D-glucose released per minute using carboxymethyl cellulose as substrate.*

Table 3: Plant growth promoting metabolites production by the soil isolates

Strain Name	**Indole acetic acid** (µg mL ± SE)	PO_4^{-3} Solubilization (µg/mL ± SE)
L3	0.454 ± 0.0003	0.071 ± 0.0003
L6	0.469 ± 0.0003	0.066 ± 0.0003
L7	0.457 ± 0.0020	0.074 ± 0.0003
L8	0.468 ± 0.0003	0.088 ± 0.0003
L9	0.429 ± 0.0003	0.066± 0.0003
L10	2.519 ± 0.0003	0.067 ± 0.0003
L11	0.443 ± 0.0003	0.065 ± 0.0003
L12	0.444 ± 0.0003	0.086 ± 0.0003
L13	0.460 ± 0.0003	0.086± 0.0003
P5	0.419 ± 0.0003	0.081 ± 0.0003
P7	2.427 ± 0.0003	0.063 ± 0.0003
P8	0.433 ± 0.0003	0.064 ± 0.0003
A1	0.374 ± 0.0003	0.066 ± 0.0003

Among the 13 isolates, 10 isolates (76.9%) have shown extracellular lipase activity. Some of the strains are also capable of producing other industrially important extracellular enzymes such as protease (all of the tested strains, 100%), amylase (L13, P7, P8, and A1; 30.7%) as well as cellulase (all of the tested strains, 100%) (Table 2). All of the 13 bacterial isolates in the present study were found to produce growth-promoting substances such as indole acetic acid (IAA) when they were grown in Luria-Bertani (LB) medium added with L-tryptophan (Table 3). All of the isolates also could solubilize inorganic phosphate (Table 3). However, none of them were positive for hydrogen cyanide production and chitinase activity.

Bacteria producing indole acetic acid (IAA) and phosphate solubilizing bacteria are capable of stimulating plant growth (Rajkumar *et al.,* 2006). Therefore, plant growth promoting bacteria (PGPB) could improve plant competitiveness and responses to external stress factors (Egamberdiyeva and Hflich, 2004). Indole acetic acid (IAA) is known to stimulate both rapid responses (e.g. increases in cell elongation) and long term responses (e.g. cell division and differentiation) in plants. L-tryptophan (L-Trp) is an amino acid, serves as a physiological precursor for biosynthesis of IAA in plants and in microbes. All of the soil isolates in the present study are found to produce growth-promoting substances like Indole acetic acid (IAA) when grown in Luria-Bertani (LB) medium amended with L-tryptophan which might help in plant growth.

Phosphorus is a key nutrient required for growth of cereal plants but less than 10% of soil phosphorus (P) enters the plant–animal cycle (De Freitas *et al.,* 1997). Consequently, P fertilizers are required in main crop production. The P in these fertilizers is rapidly reacts with the soil and becomes progressively less available for plant uptake. Under these conditions, there is, however, the prospect of using plant growth promoting bacteria (PGPB) inoscula into the crop production system, to increase cereal crops growth and yield (Pidiyar *et al*., 2004). Here, all of the isolates could solubilize inorganic phosphate, therefore they can be involve in plant growth promoting activities where they act as microorganisms that dissolve poorly soluble axed P. So, they can be termed as phosphate-solubilizing microorganisms. All of the isolates tested here showed inorganic phosphates solubilization as plant growth promoting traits. However, none of them was able to produce hydrogen cyanide and extracellular chitinase as anti-fungal phenotypes. Most of the strains tested here are capable of producing extracellular lipase along with other industrially important enzymes such as protease and cellulase. From the results obtained here, it can be concluded that these bacterial strains have potential to be used as the source of industrially important biocatalysts as well as plant growth promoting bacteria.

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