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Advances in Bioresearch

ORIGINAL ARTICLE

Exploring the Phosphorus-Enhancing Potential: Isolation, Identification, and Characterization of Phosphate-Solubilizing Bacteria from Rhizospheric Soil

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ABSTRACT
Phosphate-solubilizing microbes (PSMs) play a crucial role in converting insoluble phosphates into soluble forms, thereby enhancing phosphorus availability to plants. In this study, we aimed to isolate and identify PSMs collected from rhizospheric soil. The isolated strain was identified as Serratia based on colony characteristics and biochemical analysis. Furthermore, we explored the phosphatase activity of 77.26±0.46 U/ml, optimization of growth conditions for crude acid phosphatase was found to be temperature of 45ºC, pH was 5 and glucose and ammonium sulphate served as good substrates for carbon and nitrogen sources. Partial purification of acid phosphatase by salt precipitation and dialysis gave a yield of 36.68±0.05% compared to crude. Acetic acid was vigorously produced by the isolate, reaching concentrations of 40.41±0.35 mg/L compared to other organic acids analysis by GC-MS. The production of other plant growth-promoting enzymes and hormones were identified. The antagonistic activity as biocontrol agent of the isolate was performed on plant pathogens. These findings contribute to our understanding of microbial-driven processes for sustainable agriculture and highlight the potential application of PSMs in enhancing phosphorus availability and plant growth in agricultural systems.
Keywords: *Phosphate-solubilizing microbes (PSMs), phosphatase activity, organic acids, antagonistic activity.*

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INTRODUCTION

Phosphorus (P) is one of the most important elements in the nutrition of plants, next to nitrogen (N). It is required for all key metabolic activities in plants, including photosynthesis, energy transmission, signal transduction, macromolecular biosynthesis, respiration, and nitrogen fixation in legumes [1]. P is abundant in soil, both in its inorganic and organic forms, but because it cannot be absorbed by roots, it limits plant development. Following the frequent application of chemical fertilizers, the majority of insoluble mineral complexes containing inorganic P have been found in soil. Plants cannot absorb these insoluble precipitated forms [2] [3].

High P fixing by Al and Fe hydroxides is a prevalent concern in agricultural soils [4]. In the tropics, the soils with the highest P fixing capability cover 1,018 million hectares (ha). As a result, soil P gets fixed and, on the majority of agricultural soils, must be supplemented with artificial P fertilizers [5]. These chemical P fertilizers not only increase agricultural production costs, but they also have significant impacts on soil health and the degradation of terrestrial, freshwater, and marine resources [6]. As a result, greater P levels have been identified as a primary cause to surface water eutrophication, which can lead to algal blooms. The widespread use of chemical P fertilizers depletes soil fertility [7]. Microorganisms are an essential component of the soil P cycle because they transport P across distinct

pools of soil. Phosphate Solubilizing Microorganisms (PSM) use multiple processes of solubilization and mineralization to transform inorganic and organic soil P into bioavailable forms that plant can utilize [8]. It is critical to understand the real process of P solubilization by PSM in order to maximize the use of these microorganisms in agricultural field [9]. As a result, it is critical to better understand the plant-soil-microbial P cycle in order to reduce dependency on chemical P fertilizers. This has spurred interest in using microbes to assist P cycling in agricultural environments [10].

Several bacterial and fungal strains have been developed and thoroughly studied for their phosphate-solubilizing properties. *Pseudomonas* and *Bacillus* bacteria, as well as *Aspergillus* and *Penicillium* fungus, are common microorganisms that contribute to the transformation of P nutrients in the soil and may be employed as bio fertilizers to improve plant development and fertilization efficacy [11]. The phosphate solubilizing microorganisms (PSM) can remove phosphorous (P) from bonds with aluminium (Al), iron (Fe), calcium (Ca), and magnesium (Mg) and dissolve and make it available to plants [12]. PSM might mineralize and solubilize total soil P from both inorganic and organic pools, according to Sharma *et al*., (2013) [9]. This is due to the fact that in soil, bacterially generated organic acids have the ability to form stable complexes with cations that bind P. In addition to being capable of release fixed P, phosphate-solubilizing microorganisms offer a number of other benefits for enhancing plant development, such as the ability to produce phosphatase enzymes [13] [14]. Soils with a high organic P content may provide P availability [15]. Because plants cannot use inorganic P directly, it must first be transformed into soluble (organic) P via a mineralization process aided by soil enzymes [16].

Serratia sp. produces acid phosphatase, which is widely reported among bacterial species. *Serratia* sp. acid phosphatase has numerous uses, including plant growth stimulation [17], waste remediation and metal recovery, antagonistic action against plant diseases [18], and hydroxyl apatite biosynthesis [19]. Although there have been various publications on *Serratia* sp. phosphate solubilization and acid phosphatase production, the events of phosphate solubilization within saline mangrove soils have received less attention [20].

In recent years, many screening programs in saline environments have been carried out in attempt to isolate and characterize new enzymatic activities with features that differ from those of traditional enzymes [21]. Halophilic enzymes, in addition to being intrinsically stable and active at high salt concentrations, provide significant prospects in biotechnological applications such as food processing, environmental bioremediation, and biosynthetic processes [22] [23]. In this regard, it is crucial to identify new enzymes with optimum activity over a variety of pH values, temperatures, and salt concentrations. To improve the soil P nutrient content and maximize the P utilization, it is necessary to investigate the characteristics of PSM and their ability to increase P solubility by organic mineralization. Therefore, the present study was taken in isolating phosphate solubilizing microbes associated with rhizosphere soils and understanding its application to agricultural ecosystems.

MATERIAL AND METHODS

Soil sample collection

The rhizospheric soil samples were collected from GKVK campus, Banglore, India. Samples were stored at 4º C before processed within 24 hrs of collection.

Isolation of Phosphate Solublizing Microbes

One gram of each soil sample was added to 9 mL of phosphate buffer saline (pH 7.2), serial dilutions from 10^{-1} up to 10^{-6} were realized. Then, 100 µL of serially diluted suspension was spread on Pikovskaya media (10 g glucose, 5 g $Ca_3(PO_4)^2$, 0.2 NaCl, 0.5 g $(NH_4)SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.5 g yeast extract, 0.2 g KCl, 0.002 g $FeSO_4 \cdot 7H_2O$, 0.002 g $MnSO_4 \cdot H_2O$, 15 g agar, in 1 L distilled water (pH 7). The plates were then inoculated for 72 hrs at room temperature. The clear zone indicated the ability of the isolates to dissolve phosphate in Pikovskaya media, which contains insoluble phosphate (Tricalcium phosphate). The bacterial colonies with a clear zone were selected and purified on Pikovskaya media. The qualitative efficiency of the selected PSBs was tested according to the phosphate solubilization index (PSI), measured by the below mentioned formula. The pure isolates were kept on nutrient agar plates and stored at 4°C [24].

$$PSI = \frac{C+H}{C} \times C$$

(H = Halo zone diameter; C = Colony diameter)

Characterization and identification of selected bacterial isolates

Bacterial isolates were characterized and identified on the basis of their morphological, cultural, physiological and biochemical characteristics. The tests include gram staining, indole test, methyl red test,

nitrate reduction test, oxidase test, catalase test, voges proskauer test, sugar utilization, H_2S production and gelatin liquefaction. Identified organisms were streaked on selective media and stored at 4°C.

Acid phosphatase assay

In a 250 ml conical flask, growth medium broth from the National Botanical Research Institute (NBRIP) was used for the phosphatase extraction. The flasks were inoculated with 100 µl of bacteria. The inoculated flasks were incubated at 37°C up to 192 hrs. The samples were withdrawn at every 24 hrs and centrifuged at 10,000 rpm for 10 min at 4°C. Using the procedure described by Tabatabai and Bremner (1969), the crude acid phosphatase activity of the cell-free supernatant was measured [25].

1mL of free culture supernatant from bacterial cells was mixed with 4mL of modified universal buffer (pH 6.5). The culture supernatant was then added to 1 ml of 0.025 mM disodium p-nitrophenyl phosphate (tetrahydrate) and incubated at 37°C for 60min. To prevent microbial growth, one drop of toluene was added. After 60min of incubation, the reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$. The contents were filtered through Whatman No. 42 filter paper. The content of p-nitrophenol was evaluated in triplicate by measuring the absorbance at 420 nm using a UV-Vis spectrophotometer and extrapolating the data from a standard curve established using serially diluted p-nitrophenol solutions as reference.

Under the test conditions, one unit (U) of phosphatase activity was defined as the quantity of enzyme needed to liberate 1 mol of p-nitrophenol/ml/min from di-Na p-nitrophenyl phosphate (tetrahydrate) [26].

Optimization of growth condition parameters

Optimization of crude acid phosphatase production was carried out in triplicate in NBRIP broth under different conditions: temperature 25 to 65°C with 10°C increments; pH 3.0 to 10.6; carbon sources, including lactose, maltose sucrose, and glucose; and nitrogen sources, including ammonium molybdate, urea, potassium nitrate and ammonium sulphate. After 48hrs of incubation phosphatase activity was determined.

Partial purification of acid phosphatase

Partial purification of crude phosphatase was carried out by salt precipitation (ammonium sulphate) up to 70% followed by overnight dialysis [27]. Quantification of protein content of crude and partially purified phosphatase was carried out following the method of Lowry *et al* (1951), with Bovine serum albumin as a standard [28].

Organic Acid Analysis by GC-MS

The isolate was cultured in NBRIP broth medium for acid organic determination. Before autoclaving, 50 mL of NBRIP broth medium was adjusted to pH 7.0 in a 250 mL flask. The medium was inoculated with 200 µL of fresh inoculum (1.8×10^8 CFU/mL) and incubated in shaking conditions at 120 rpm/min at 30 °C for 72 hrs. After centrifuging the sample for 5 to 10 min, 5 mL of supernatant was transferred to a Falcon tube. The pH of the sample was measured and 100 µL of sulfuric acid (1 N) was added. The pH was kept between 2 and 4. The samples were filtered through a 0.2 µm filter and then phenol (0.05 M) was added. Gas Chromatography coupled Mass Spectrometry (GC-MS) was used to determine the presence of organic acids (acetic acid, formic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, caproic acid, heptanoic acid) in the samples. The calibration curves of the standards were used to quantify the acids [29].

Production of plant growth promoting hormones and enzymes

Indole-3-acetic acid (IAA)

Based on their capacity to solubilize P, the selected phosphate solubalizing bacteria (PSB) were analyzed for IAA production. 200 µl of fresh bacterial cultures were inoculated in 30 mL of Luria-Bertani broth containing 0.1% L-tryptophan, and they were incubated in incubator Shaker at 28°C and 140 rpm/min for 72 hrs in dark. The bacterial sample was centrifuged at 10,000 rpm for 10 min. 2ml of supernatant was combined with Salkowski reagent (2% of 0.5M ferric chloride in 35% perchloric acid) after centrifugation. The optical density at 530 nm was determined using a UV spectrophotometer after 30 minutes in the dark for pink colour development. The amount of IAA produced was calculated by the standard graph of pure indole acetic acid [30].

Aminocyclopropane-1-carboxylate (ACC) deaminase

The ACC deaminase activity was determined as described minimal salts medium, which contains (per liter): 4 g KH_2PO_4, 6 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 2 g glucose, 2 g gluconic acid and 2 mg citric acid with trace element solution (1 mg $FeSO_4.7H_2O$, 10 µg H_3BO_3, 11.19 µg $MnSO_4.H_2O$, 124.6 µg $ZnSO_4.7H_2O$, 78.22 µg $CuSO_4.5H_2O$ and 10 µg MoO_3). Filter sterilized ACC solution (3 mM) was applied to the agar plates, dried for 10 minutes, and then inoculated with bacterial strains. Observation of the growth made after 2 days incubation at 28°C as described earlier.

Hydrogen Cyanide Production

Selected isolate was streaked on nutrient agar amended with 0.44% glycine. Following that, a filter paper soaked in 2% sodium carbonate and 0.5 mL picric acid solution was inserted in the agar plate lid and covered with parafilm. The plates were incubated for 4 days at 28°C until the plates turn yellow color to the brown and considered as positive.

Siderophore

Production of siderophore by the strains was determined using the $FeCl_3$ test and the chrome azurol S agar assay. Briefly, 10 µl of bacterial isolate was dropped onto the center of a CAS plate. After incubation at 25°C for 3 days, siderophore production was assessed on the basis of change in color of the medium from blue to orange [31].

Production of beneficial enzymes

Protease

Skim milk agar medium was used to test protease activity, which contains 5 g pancreatic digest of casein, 1 g glucose, 2.5 g yeast extract, 7% skim milk solution, and 20g agar in 1 L distilled water (pH 7). Isolate was inoculated for 2 days at 28°C, proteolytic activity was identified by clear zone around the isolate [32].

Chitinase

The chitinase activity of the isolate was screened on chitin agar medium, which contains 1.62 g nutrient broth, 0.5 g NaCl, 6 g, 8 g colloidal chitin and 15 g agar in 1 L distilled water (pH 7). Bacterial cells were spot inoculated and after 5 days incubation at 30°C, chitinase activity was identified by clear zone around the cells [33].

Cellulase

By growing bacterial isolates onto M9 medium agar supplemented with 10 g of cellulose and 1.2 g of yeast extract per liter, cellulase production was tested. After 8 days of incubation at 28°C, colonies surrounded by clear halos were considered positive for cellulase production [34].

Pectinase

The Pectinase production was determined using medium amended with 4.8 g of pectin per liter. After 2 days of incubation at 28°C, plates were flooded with 2 mol l-1 HCl and strains surrounded by clear halos were considered positive for pectinase production [35].

Antagonistic effect

The method used to test antagonism against fungal pathogens using the dual culture method. Pathogenic fungi in circular blocks measuring 6 mm were grown on the test potato dextrose agar (PDA) media. Meanwhile, the test bacteria isolate were grown on PDA media with a streak technique of 3 cm. The distance between the bacterial isolate and the test organism was 3 cm each. The antagonism test treatment was observed for nine days and incubated at room temperature (28 °C). The growth reduction was calculated using:

% growth inhibition= $\frac{Rc-Rt}{Rc} \times 100$

R_c is colony diameter of pathogen (control) and R_t is colony diameter of pathogen inoculated with tested fungi [36].

Statistical Analysis

All the experiments were performed in triplicates and Statistical Package for the Social Sciences (SPSS) was used to interpret the following data in form of mean ($\bar{x}$) ± standard deviation (SD).

RESULTS AND DISCUSSION

Isolation and identification of Phosphate solubilizing microbe (PSM):

From the rhizosphere soil sample, 20 bacteria were recovered. On Pikovskaya media supplemented with $Ca^3(PO_4)^2$ as the sole P source, all of the isolates were tested for phosphate solubilizing activity. The isolates that produced P solubility index (PSI) greater than 2 cm on plates were selected; 6% of the strains (PSM 2, PSM 3, and PSM 14) that demonstrated phosphate solubilizing activities (Table 1). Several authors have focused their studies on the solubilization of Ca^3 $(PO_4)^2$, $FePO_4$, and $AlPO_4$ [37] [38]. The isolates tested in our study were able to solubilize Ca^3 $(PO_4)^2$; however, solubilization of $FePO_4$ and $AlPO_4$ was minimal when compared to Ca^3 $(PO_4)^2$; additionally, the selected isolates demonstrated high efficiency of solubilization of Ca^3 $(PO_4)^2$ because $FePO_4$ and $AlPO_4$ have a more complex structure than Ca^3 $(PO_4)^2$. Previous research found that Ca^3 $(PO_4)^2$ had the maximum solubilization. Similarly, Devi and Thakuria (2021) discovered in their study of phosphate solubilizing bacteria (PSB) predominance in rice rhizospheric soils that only 40.7% of 172 isolates dissolved aluminium phosphate [37].

The selected PSM strains were all gram negative. PSM2 colonies appeared shiny, red, and with round and smooth margins. PSM 3 was greenish, a little wet, transparent, and shiny. PSM 14 was round but smooth, and they all had the same shapes in morphology. The organisms were identified as *Serritia*, *Pseudomonas* and *Klebsiella* when subjected to gram staining (Fig. 1), biochemical tests as per Bergey's manual of systematic bacteriology (Table. 2).

Isolates	Colony Diameter (cm)	Halo Zone Diameter (cm)	PSI = C + H/C
PSM 2	0.77±0.02	2.6±0.11	4.34±0.02
PSM 3	0.97±0.02	2.3±0.15	3.37±0.02
PSM 14	0.94±0.05	2.8±0.26	4.05±0.05

Table 1: Phosphate solubilization index (PSI) of the selected PSM isolates.

Previous studies found phosphatase-coding genes in *Serratia marcescens* and *Pseudomonas* [39]. Furthermore, Lavania and Nautiyal (2013) discovered that the soil isolate *S. marcescens* NBRI1213 is an effective phosphate solubilizer as well as a possible plant growth promoter [40]. *Serratia* and *Alcaligenes faecalis* show antagonistic action against plant diseases and can create hydroxyl apatite in addition to phosphate solubilization [41]. The detected isolates therefore had evidence that they were PSM (Table. 1).

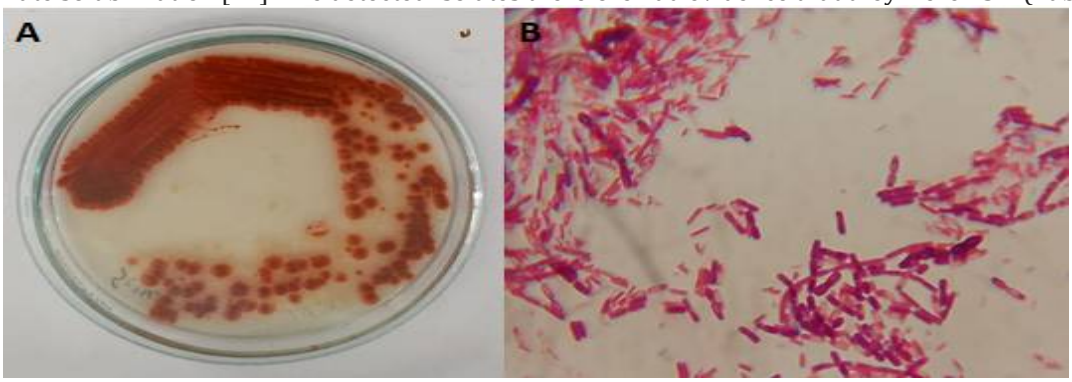

Fig. 1. Results for colony morphology and gram staining. A. red pigmented colony on nutrient medium; B. gram negative rod shaped bacillus was observed under 100x

Phosphatase assay

Serratia sp inhabiting soil produces significant level of acid phosphatase. In the current investigation, the maximal acid phosphatase production by *Serratia* sp. was reported after 48 hrs of incubation (Table. 3). Several studies have also observed substantial acid phosphatase activity from *Serratia* sp. and other bacterial genera [42]. In addition to the current study, phosphate solubilizations in various ranges have been reported from other mangrove habitats. Seven bacterial species isolated from Chollangi, East Godavari mangrove soil, including two *Bacillus subtilis*, three *Pseudomonas* sp., and two *Azotobacter* sp., shown phosphate solubilizing capacity in the range of 80-100 mg/l. Kathiresan and Selvam (2006), isolated 24 phosphate solubilizing bacteria from the mangrove soils of the Vellarestuary at Parangipettai, southeast coast of India, whose phosphate solubilizing efficiency was in the range of 0.012–0.141 mg/l [43]. Therefore, the present isolate has better efficiency is phosphate solubilization.

Biochemical test	PSM – 2	PSM – 3	PSM - 14
Indole test	-	-	-
MR test	-	+	-
VP test	+	-	+
Citrate test	+	+	+
Catalase test	+	+	+
Oxidase test	-	+	-
Glucose	+	+	+
Fructose	+	+	+
Maltose	+	+	+
Sucrose	+	+	+
Mannitol	+	-	-
Hydrogen sulphide test	-	-	-
Urea test	-	+	+
Pigmentation	Red	Green	White

Table 2: Results for biochemical tests. PSM-2 was identified was *Serritia*, PSM-3 has *Pseudomonas* and PSM-14 was identified as *Klebsiella*

Isolate code	Organism	Phosphatase activity (U/ml)
PSM 2	*Serratia*	77.26±0.46
PSM 3	*Pseudomonas*	63.76±0.68
PSM 14	*Klebsiella*	70.1±0.41

Table 3: Results for Phosphatase activity on selected PSM isolates.

Optimization of growth condition parameters for crude acid phosphatase production

Phosphatase production is greatly influenced by growth parameters such as different pH, temperature, carbon source and nitrogen source (Fig. 2). To optimise crude enzyme production, the optimal condition of these parameters under laboratory conditions will be evaluated. *Serratia* having high phosphate solubaliziation ability compared to others, it was subjected to optimization study.

The effect of temperature on enzyme activity is a critical parameter which usually varies from organism to organism [44]. According to Frankena *et al.,* (1986), there is a relationship between enzyme production and energy metabolism in bacteria, which is controlled by temperature and oxygen absorption [45]. Because temperature influences the synthesis of extracellular enzymes, modifying the physical features of the cell membrane may impact their secretion. The greatest acid phosphatase activity of *Serratia* was recorded at 45°C in the current investigation. Acid phosphatase activity in the same range was also reported earlier [46] Increases or decreases in pH above the optimal range can impact the active site of amino acids by preventing the enzyme from forming an enzyme substrate complex, resulting in a reduction in enzyme activity. The isolate under investigation was discovered to synthesize acid phosphatase best at pH-5.0. Similar observations have been reported for the acid phosphatases from *Serratia* isolated from soil of mangrove forest [47].

The use of low cost substrates for the production of industrial enzymes is one of the alternative ways to reduce production costs significantly [48]. The amount of enzyme produced by each substrate differs depending on the type of carbon and nitrogen source preferred by organisms [49]. The influence of carbon and nitrogen source on enzyme synthesis was studied, and it was discovered that not all carbon and nitrogen sources would work as an enhancer for enzyme production [50]. The bacterial isolate *Serratia* produced the most acid phosphatase in medium supplemented with glucose as a carbon source and ammonium sulphate as a nitrogen source, according to the present results.

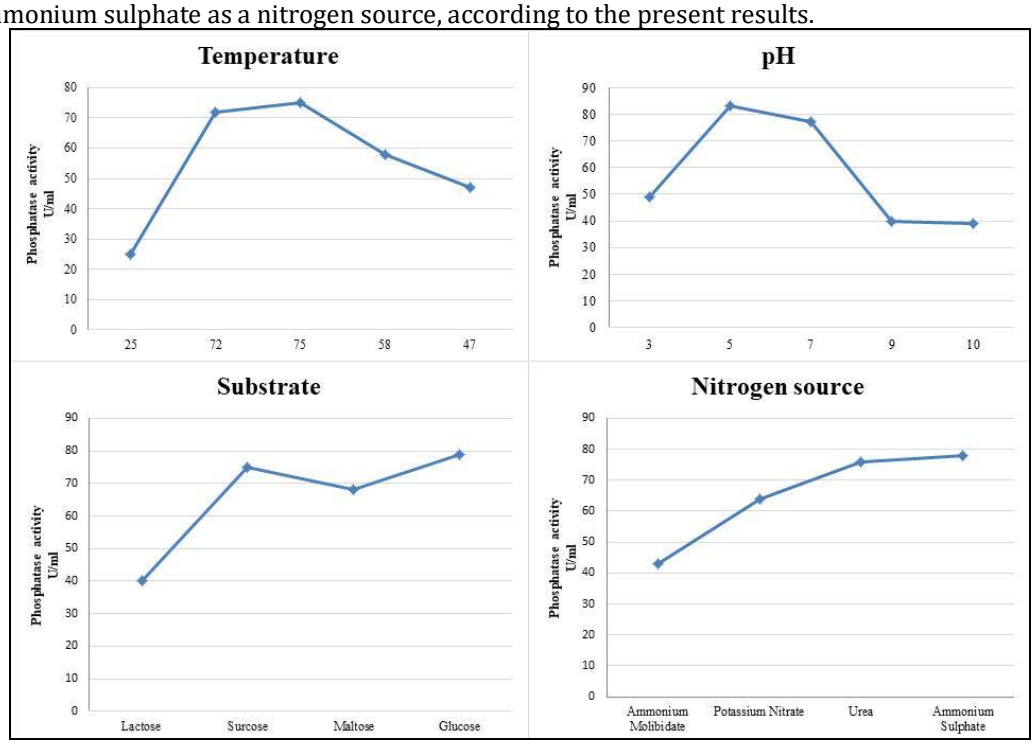

Fig. 2. Results for Optimization of crude acid phosphatase production

Partial purification of acid phosphatase

In order to study the catalytic activity of enzymes and develop applications for their use in applied sciences and industry, there is need to purify and characterize the enzyme [51]. The purification process may separate the protein and non-protein parts of the mixture by downstream processing [52]. In the

present study, the crude enzyme solution was partially purified up to 1.49±0.02 fold with a total yield and specific activity of 36.68±0.05% and 9.58±0.02 U/mg respectively (Table. 4).

Isolate PSM 2	Total Volume (ml)	Protein concentration (mg/ml)	Total Protein content (mg)	Phosphatase activity (U/ml)	Total phosphatase activity (U)	Specific activity (U/mg)	Fold of purification	Total yield (%)
Crude	49.6 ±0.57	11.83±0.28	599±0.57	77.4±0.40	3840±0.57	6.4±0.05	0.96±0.05	99.83±0.28
Salt precipitation and dialysis	14.83±0.28	9.0±0.11	135±0.30	86.23±0.16	1293.45±0.50	9.58±0.02	1.49±0.02	36.68±0.05

Table 4: Results of partial purification of crude acid phosphatase.

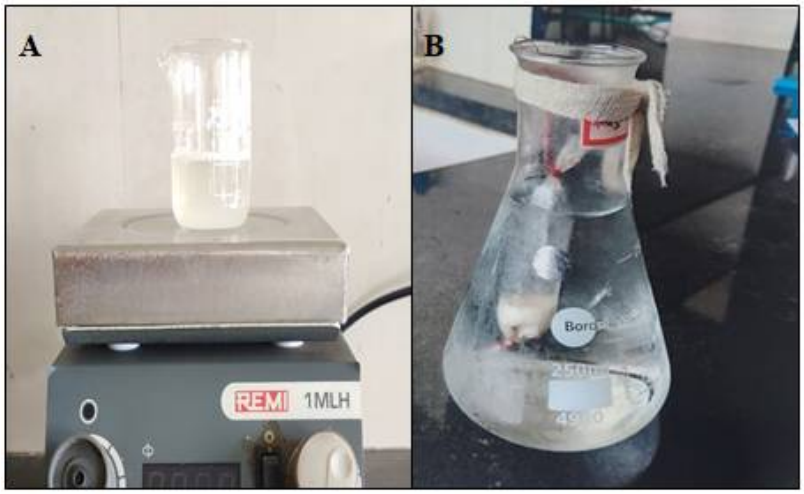

Fig. 3. Results for partial purification. A. Salt precipitation; B. Dialysis

Organic Acid Analysis by GC-MS

Organic acids increases Phosphorus (P) availability by blocking P absorption sites on soil particles or by forming complexes with cations on the soil mineral surface As a result, the presence of organic acids was detected by GC-MS (Fig. 4). Acetic acid was vigorously generated by the isolate, reaching concentrations of 40.41±0.35 mg/L. They also produced formic, propionic, isobutyric, butyric, isovaleric, caproic, and heptanoic acids, with considerable different concentration (Table. 5).

Organic acids have different abilities to release P bonds. Glucose is the principal element of NBRIP medium, and phosphate solubilizing microorganisms prefer glucose as a carbon source to produce organic acids [53]. Both in plants and microorganisms, the primary mechanisms of P solubilization are H^+ excretion, organic acid production, and acid phosphatase biosynthesis [54]. Organic acids, including acetate, lactate, malate, oxalate, succinate, citrate, gluconate, ketogluconate, can form complexes with the iron or aluminium, thus releasing plant available phosphate into the soil [55].

Isolate code	Acetic	Formic	Propionic	Isobutyric	Butyric	Isovaleric	Caproic	Heptanoic
	mg/L							
PSM 2	40.41±0.35	3.98±0.02	1.59±0.01	6.86±0.03	8.51±0.01	1.32±0.05	0.59±0.02	0.56±0.05

Table 5: Quantification of organic acids produced by PSM 2 strains in liquid NBRIP culture medium

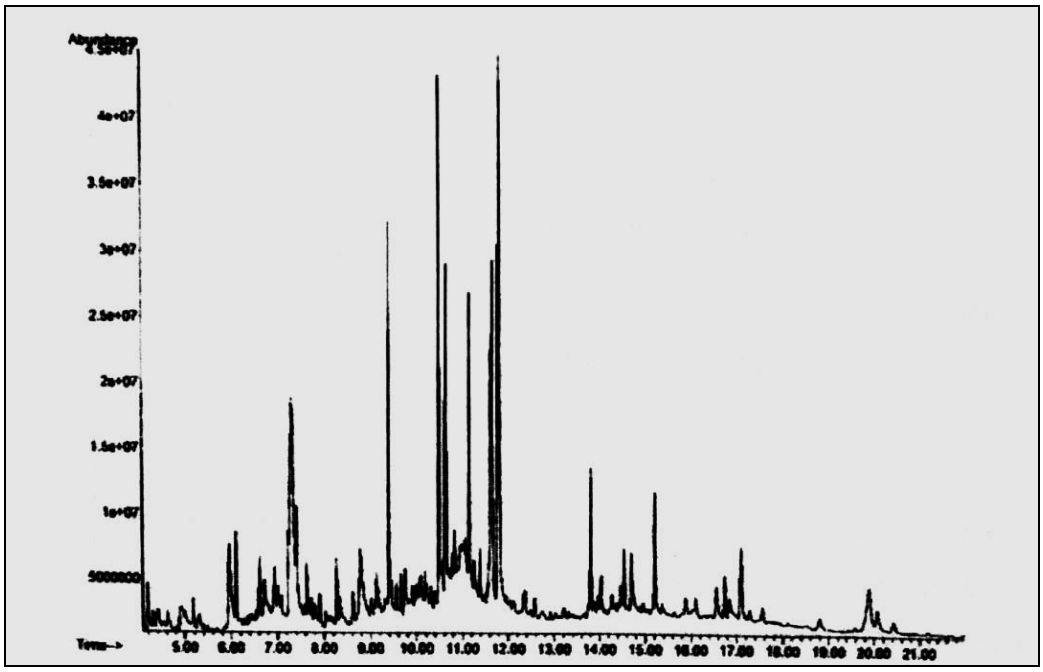

Fig. 4. Results for organics acid analysis by GS-MS

Production of other plant growth promoting enzymes and hormones

Indole-3-acetic acid (IAA)

In addition to their ability to solubilize inorganic phosphates, PSB efficiency is due not only to their potential to raise P availability, but also to their capacity to produce growth-regulating agents such as IAA, a growth regulator that aids in cell growth and division, stress resistance, root lengthening, nitrogen fixation stimulation, and biosynthesis of various metabolites [56]. The selected isolate produced 68.80±0.39 µg/ml of IAA (Fig. 5). The results co related with the work of Hasuty *et al.*, 2018 were the highest IAA concentration was produced by *S. marcescens subsp. marcescens* strain KB01, IAA concentrations of 64.75 g/mL, 56.60 g/mL, and 18.06 g/mL were found in *S. marcescens subsp. marcescens* strain KB05, and in *R. aff. qingshengii* strain 100A, respectively [57].

Fig. 5. Results for IAA production. T is test and C is control

Aminocyclopropane-1-carboxylate (ACC) deaminase

Rhizobacteria known as plant growth promoting rhizobacteria (PGPR) with 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity have the ability to support plant growth and development under adverse conditions. In plants, ACC serves as the hormone ethylene's direct precursor. According to several reports, certain PGPR have an enzyme called ACC deaminase that may break down ACC into ammonia and α-ketobutyrate, which lowers the quantity of ethylene in the plants [58]. The present isolate PSM 2 that is *Serratia* sp gave zone of by producing ACC deaminase indication positive result. In the early reports highest ACC deaminase activity was identified as *Serratia marcescens* strain JW-CZ2 [59]. Another study found that the development of coconut palms improved after being inoculated with the bacteria *S. marcescens* KiSII, which has ACC deaminase activity [60].

Fig. 6. Positive result for Aminocyclopropane-1-carboxylate (ACC) deaminase synthesis

Hydrogen Cyanide Production (HCN)

The biological control of pathogens is thought to be significantly influenced by the synthesis of HCN by rhizobacteria. It is volatile, a secondary metabolite that inhibits the growth of pathogen due to inhibitor of metal enzymes, particularly cytochrome C oxidases in an electron transport system. In this study, the present isolate PSM 2 that is *Serratia* was able to produce HCN and turning the medium into brown. Hence, there are many reports stating *Serratia* sp are unique PSB and can produce HCN in adequate amount to control microbial infections in plants [61].

Fig. 7. Positive result for Hydrogen Cyanide Production

Siderophore

Microorganisms and plants that flourish in low iron environments create siderophores, which are organic molecules with low molecular weights. It is produced by phosphate solubalizing bacteria that facilitate the plant growth by providing iron to plants. In the present study, siderophore production was carried out on solid CAS blue agar where the present isolate *Serratia* developed orange halo circle around the colonies represents iron chelation in the medium. These results are consistent with Aliyat *et al.*, 2022, who found *Serratia* sp to be a distinct producer of siderophore [24]. Additionally, in a work by Schneider *et al.,* 2020 experiment showed that *Serratia* sp. produces greater siderophore content in conditions of iron deficiency [62].

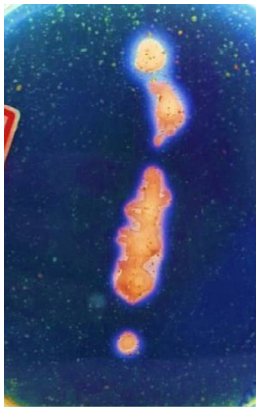

Fig. 8. Result for siderophore production on CAS medium

Production of beneficial enzymes

Production of antifungal metabolites by bio control agents is strongly correlated with their biocontrol activity. Chitinases, which were previously thought to be important biocontrol factors together with other hydrolytic enzymes, destroy fungal cell walls and suppress fungal development [63]. *Serratia* is a competent producer of chitinases, with well-studied production, activity, and gene regulation (Gutierrez-Roman *et al.,* 2014). Therefore, in the present study the isolate *Serratia* produced protease, chitinase, cellulose, and pectinases during qualitative analysis. Studies have shown the potentiality of PSB strains to be used as bio control agents and bio-fertilizers for agriculture crop and soil improvement by production of such enzymes [64]. Purified chitinases from *S. plymuthica, S. proteamaculans*, and *S. marcescens*, are reported to be antagonistic to different pathogenic fungi [65]. But in spite of the strong antifungal activity, factors other than chitinases are considered more essential for biocontrol of plant diseases indicating that chitinases may form only a part of the multi-component mechanism which underlies biocontrol by *Serratia* strains in the environment [66].

Organism	Protease	Chitinase	Cellulose	Pectinases
PSM-2	**+**	**+**	**+**	**+**

Table 6: Results for beneficial enzyme synthesis

Antagonistic effect

Ecofriendly biocontrol agents to control pathogenic fungi are in demand globally. The synthesis of various antibiotics/antifungals is the main characteristic of PGPB, which is most often associated with the ability of bacteria to prevent the proliferation of plant pathogens, especially fungi. In the present study, the highest percentage of inhibition against *F. solani* (65± 0.35%), *G. candidum* (52± 0.11 %) and *A. alternata* (70± 0.15%) was shown. *Serratia* inhibited mycelial growth by 75.5 0.80% against *Fusarium foetens* NCIM 1330 in a prior study [67]. Addition to that a pigment producing strain of *Serratia* sp. isolated from the rhizosphere of *Bacopa monnieri* (L.) was found to have broad spectrum antifungal activity against various phytopathogens [68].

Organism tested	Antagonistic percentage (%)
F. solani	65± 0.35
G. candidum	52± 0.11
A. alternata	70± 0.15

Table 7: Antagonistic percentage on plant pathogen

CONCLUSION

Phosphorus (P) is an important macronutrient for plant development. As a result, in intensive agriculture, phosphorus in the form of fertilizer is critical to achieving high yields. Because degradable natural phosphorus supplies are limited, agriculture requires a tailored and ecologically beneficial supply. Phosphate-solubilizing microbes (PSMs) play a crucial role in converting insoluble phosphates into soluble forms, thereby enhancing phosphorus availability to plants. In this study, our aim was to isolate and identify PSMs collected from rhizospheric soil. Among 20 isolates, *Serratia* was identified based on biochemical, selective media techniques and had more phosphate solubilizing index. Quantitative estimation of phosphatase revealed that 77.26±0.46 U/ml of enzyme activity. The optimized growth conditions for crude acid phosphatase production were determined by assessing the effects of various parameters, including pH, temperature, carbon, and nitrogen sources. The results for optimization of

growth conditions for crude acid phosphatase was found to have temperature of 45 ºC, pH was 5 and glucose and ammonium sulphate acted as a good substrates for carbon and nitrogen. In addition acid phosphatase was partially purified using salt precipitation and dialysis techniques to obtain purified crude enzyme for commercial use. Partial purification of acid phosphatase gave an yield of 36.68±0.05% compared to crude. To gain insights into the mechanism of phosphate solubilization, organic acid analysis was carried out using GC-MS. The identified organic acids were found to play a vital role in phosphate solubilization, Acetic acid reached concentrations of 40.41±0.35 mg/L compared to other organic acids analysis. Furthermore, the isolated PSMs were evaluated for the production of other plant growth-promoting enzymes and hormones, such as indole-3-acetic acid (68.80±0.39 µg/ml), siderophores, hydrogen cyanide production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. The production of these compounds can potentially enhance plant growth and development by promoting nutrient uptake and reducing stress responses. PSM secrets certain secondary metabolites that act as biocontrol agents on plant pathogens. In the present study, the highest percentage of inhibition against *F. solani* (65%), *G. candidum* (52%) and *A. alternata* (70%) was seen. These findings contribute to our understanding of microbial-driven processes for sustainable agriculture and highlight the potential application of PSMs in enhancing phosphorus availability and plant growth in agricultural systems.

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