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ORIGINAL ARTICLE

Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India

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Abstract Phosphorus is an essential element for all life forms. Phosphate solubilizing bacteria are capable of converting phosphate into a bioavailable form through solubilization and mineralization processes. Hence in the present study a phosphate solubilizing bacterium, PSB-37, was isolated from mangrove soil of the Mahanadi river delta using NBRIP-agar and NBRIP-BPB broth containing tricalcium phosphate as the phosphate source. Based on phenotypic and molecular characterization, the strain was identified as *Serratia* sp. The maximum phosphate solubilizing activity of the strain was determined to be 44.84 μg/ml, accompanied by a decrease in pH of the growth medium from 7.0 to 3.15. During phosphate solubilization, various organic acids, such as malic acid (237 mg/l), lactic acid (599.5 mg/l) and acetic acid (5.0 mg/l) were also detected in the broth culture through HPLC analysis. Acid phosphatase activity was determined by performing p-nitrophenyl phosphate assay (pNPP) of the bacterial broth culture. Optimum acid phosphatase activity was observed at 48 h of incubation (76.808 U/ml), temperature of 45 °C (77.87 U/ml), an agitation rate of 100 rpm (80.40 U/ml), pH 5.0 (80.66 U/ml) and with glucose as a original carbon source (80.6 U/ml) and ammonium sulphate as a original nitrogen source (80.92 U/ml). Characterization of the partially purified acid phosphatase showed maximum activity at pH 5.0 (85.6 U/ml), temperature of 45 °C (97.87 U/ml) and substrate concentration of 2.5 mg/ml (92.7 U/ml). Hence the present phosphate solubilizing and acid phosphatase production activity of the bacterium may have probable use for future industrial, agricultural and biotechnological application.

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1. Introduction

Mangrove forests inhabit the tropical and subtropical regions of the world, thriving in the transitional regions between the land and the sea and offering a unique environment for diverse groups of organisms [15]. These ecosystems are characterized by periodic tidal flooding, which results in a highly saline soil profile, with variable levels of nutrients. Muddy mangrove soils have a large capacity to absorb nitrates and insoluble phosphates carried by the tides [52]. Phosphorus usually precipitates due to the abundance of cations in the interstitial waters surrounding mangrove sediment, making it non-bioavailable to plants and most other organisms.

Phosphorus is a structural component of nucleic acids, rendering it an essential macronutrient for all life forms. Soil phosphate solubilizing bacteria are capable of converting phosphate into a bioavailable form through solubilization and mineralization processes, which in turn supply soluble phosphorus to plants [42]. Microorganisms capable of dissolving low solubility CaPs are termed as mineral phosphate solubilizers (MPS) [13]. These insoluble phosphates are converted into soluble forms through the process of acidification, chelation, exchange reactions and production of organic acid by phosphate solubilizing microorganisms (PSMs) [10]. This process not only mobilizes the fertilizers added to soil, but also compensates for higher cost of manufacturing of fertilizers in industry. Solubilization of phosphate-rich compounds is also carried out by the action of phosphatase enzymes called acid phosphatases (AcPase). Acid phosphatases (EC 3.1.3.2) are a ubiquitous class of enzymes that catalyse the hydrolysis of phosphomonoesters at an acidic pH. In all bacteria, these enzymes catalyse the hydrolysis of a wide variety of phosphomonoesters and catalyse transphosphorylation reactions by transferring a phosphoryl group to alcohol in the presence of certain phosphate acceptors [11]. Bacteria possess acid phosphatase enzymes located in the bacterial cell wall and in the extracellular polymeric substance (EPS) that surrounds it [44]. Among the bacterial species, acid phosphatase production by *Serratia* sp. is very well documented. Acid phosphatase produced by *Serratia* sp. has several applications, including plant growth promotion [46,14], waste remediation and metal recovery [27], antagonistic activity against plant pathogens [9] and hydroxyl apatite biosynthesis [44]. Although there are several reports on phosphate solubilization and acid phosphatase production by *Serratia* sp. [44,7,3,48], events of phosphate solubilization within saline mangrove soils is relatively less explored [24,40,50]. In recent years, different screening programs have been performed in saline habitats in order to isolate and characterize novel enzymatic activities with different properties to those of conventional enzymes. Besides being intrinsically stable and active at high salt concentrations, halophilic enzymes offer important opportunities in biotechnological applications, such as food processing, environmental bioremediation and biosynthetic processes. In this sense, the finding of novel enzymes showing optimal activities at various ranges of salt concentrations, temperatures and pH values is of great importance [18]. With the above notions in consideration, this study focuses on phosphate-solubilizing bacterium which has been identified and characterized from saline mangrove soils of the Mahanadi river delta, Odisha, India. Further attempt has been made to purify and characterize the acid phosphatase enzyme produced by the bacterial isolates which may have potential biotechnological application.

2. Materials and methods

2.1. Bacteria isolation and biochemical characterization

Phosphate solubilizing bacteria were isolated from soil samples obtained from different locations within the mangrove forests of the Mahanadi river delta, Odisha, India. One gram of soil sample is serially diluted and pour plated in triplicates on plates containing National Botanical Research Institute's phosphate (NBRIP) agar, containing l^{-1}: glucose 10 g: $Ca_3(PO_4)_2$ 5 g; $MgCl_2{\cdot}6H_2O$ 5 g; $MgSO_4{\cdot}7H_2O$ 0.25 g; KCl 0.2 g and $(NH_4)_2SO_4$ 0.1 g; pH-7.0 [34]. Colonies forming clear surrounding halos was selected as phosphate-solubilizing bacteria and named as PSB. The isolated PSB was also screened in triplicate for their ability to render the blue colour of the NBRIP – bromophenol blue broth medium (pH-7.0) colourless, due to the formation of organic acid and a decrease in pH [29]. We determined culture characteristics, such as colony appearance, spore formation, and motility of each strain according to standard methods. Cell shape and size were determined by scanning electron microscopy SEM) (Zeiss, Sigma). Oxidase, catalase, urease production, indole production, nitrate reduction, citrate utilization, glycerol utilization, methyl red, Voges–Proskauer (V–P) reaction and acid–gas production from sugar were also tested. Hydrolysis of starch, tributyrin, Tween-80, cholesterol, gelatine, casein, pectin, and chitin by the culture were also determined. The antibiotic sensitivity test of the isolate was carried out against norfloxacin, gentamycin, chloramphenicol, ciprofloxacin, and ampicillin. The salt tolerance and thermal tolerance capacity of the isolates were checked by growing them in broth medium supplemented with different concentrations of salt ranging between 2% and 12%, and in different incubation temperatures ranging from 30 °C to 60 °C. The results were compared with Bergey's Manual of Determinative Bacteriology [20].

2.2. Molecular identification of the bacterial strain

The 16S rRNA gene of the phosphate solubilizing bacterium was amplified using universal 27F forward primer (5′AGGC CTAACACATGCAAGTC-3′) and 1492R reverse primer (5′GGGCGGWGTGTACAAGGGC- 3′), as described by Das et al. [12]. PCR product was purified using QIAquick@ gel extraction kit, QIAGEN, GmbH (Germany) and nucleotide sequence were determined using the BigDye Terminator v 3.1 Cycle sequencing kit in an automated 3130xl Genetic Analyzer System (Applied Biosystems, HITACHI, USA). Forward and reverse sequences were aligned using seqscape software (Applied Biosystems, USA) to get the final sequence (585 bp). The final partial sequence was submitted to NCBI gene bank in order to obtain the corresponding accession number. A phylogenetic tree was constructed using MEGA 4.0 software, using the neighbour- joining DNA distance algorithm with a bootstrap of 1000.

2.3. Quantitative estimation of soluble phosphate

Erlenmeyer flasks containing 100 ml of NBRIP broth without bromophenol were inoculated with the phosphate solubilizing bacterial isolates in triplicate. Non-inoculated medium served as a control. The flasks were incubated in an incubator shaker at 37 °C up to 264 h, at a shaking speed of 100 rpm. The pH of the culture medium was measured at specific time intervals. 5 ml of bacterial culture samples was collected at every 24 h and centrifuged at 10,000 rpm for 10 min. The supernatant was separated from the bacterial cells by successive filtration through Whatman No.1 filter paper followed by 0.22 µm Millipore membrane, and used to estimate phosphate released spectrophotometrically (880 nm) in triplicate according to the standard method of Murphy and Riley [31].

2.4. Determination of organic acid produced by the bacterium

For analysis of organic acids, the bacterial isolate was inoculated in a 250 ml conical flask containing 50 ml of NBRIP broth medium in triplicate. The flasks were incubated at 37 °C temperature in an orbital shaker at 100 rpm for 144 h. One millilitre of incubated sample was centrifuged at 10,000 rpm (Mikro-200, Hettich Zentrifugen, Germany) for 15 min. and filtered through 0.22 µm nylon membranes (Pall India Pvt. Ltd.) to obtain cell-free culture supernatant. Twenty microlitres of filtered supernatant was injected to HPLC (LC-10AT, Shimadzu). The organic acid separation was carried out on an ion exclusion column Aminexs@ HPX-87H 300 mm X 7.8 mm (Bio- Rad Laboratories Inc.) with 0.008 M H_2SO_4 as mobile phase at a constant flow rate of 0.6 ml/min and at operating temperature of 30 °C. The retention time of each test signal was recorded at a wavelength of 210 nm (SPD 10A, Shimadzu) and compared with organic acid analysis standard kit (Bio-Rad Laboratories Inc.) following the methods of Yadav et al. [56]. Organic acid standard kit of Bio-Rad Corporation contained no lactic acid, therefore lactic acid (Sigma, USA) was injected separately.

2.5. Acid phosphatase assay

Extraction of AcPase was carried out using 100 ml of sterilized NBRIP broth in a 250 ml conical flask. The flasks were inoculated with 100 µl of bacterial culture in triplicate. The inoculated flasks were incubated at 37 °C up to 192 h. The samples were withdrawn at every 24 h and centrifuged at 10,000 rpm for 10 min at 4 °C. The cell-free supernatant was assayed for crude acid phosphatase activity according to the method outlined by Tabatabai and Bremner [49]. 1 ml of bacterial cell free culture supernatant was mixed with 4 ml of modified universal buffer (pH 6.5). Further 1 ml of 0.025 mM disodium *p*-nitrophenyl phosphate (tetrahydrate) was mixed with the culture supernatant and incubated at 37 °C for 1 h. One drop of toluene was added to stop the microbial growth. After 1 h 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 concentration of *p*-nitrophenol was measured in triplicate by measuring the absorbance at 420 nm using UV–Vis spectrophotometer and extrapolated the values on standard curve determined using serially diluted solutions of *p*-nitrophenol as standard. One unit (U) of phosphatase activity was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenol/ml/min from di-Na *p*-nitrophenyl phosphate (tetrahydrate) under the assay condition.

2.6. Optimization of growth condition parameters for crude acid phosphatase production

Optimization of crude acid phosphatase production was carried out in triplicate by culturing the bacterial isolates for 48 h in NBRIP broth under different conditions: temperature 25–65 °C with 10 °C increments; shaking rates of 0, 60, 80, 100 and 120 rpm; pH 3.0–10.6; carbon sources, including lactose, sucrose, maltose and glucose; and nitrogen sources, including ammonium molybdate, potassium nitrate, urea and ammonium sulphate. Phosphatase production was determined according to the method of Tabatabai and Bremner [49].

2.7. Partial purification of acid phosphatase

Partial purification of crude acid phosphatase was carried out by ammonium sulphate precipitation up to 70% saturation followed by overnight dialysis [45]. Quantification of protein content of crude and partially purified phosphatase was carried out following the method of Lowry et al. [26] with Bovine serum albumin as a standard (Sigma, Germany).

2.8. Characterization of partially purified acid phosphatase

The impact of parameters, such as pH from 3 to 10.6 (by adjusting the pH of the buffer), temperature (at 10 °C intervals) from 25 to 65 °C and different substrate concentrations (p-nitrophenyl phosphate) from 0.5 mg to 2.5 mg ml^{-1} were studied in triplicate for characterization of partially purified acid phosphatase activity following the method of Tabatabai and Bremner [49].

2.9. Statistical analysis

An ANOVA for multiple comparisons was performed using Graph Pad Prism version 5.01. A P-value $\leqslant 0.05$ was considered to be significant.

3. Results

3.1. Isolation and identification of bacteria

Phosphate solubilizing bacteria were isolated from the mangrove soil of Mahanadi river delta, in terms of the formation of halo zones on NBRIP-agar medium and change of intensity of colour of bromophenol blue of NBRIP-BPB broth medium. The bacterium isolated was named as PSB-37. Based on the morphological and biochemical analysis, the bacterium, PSB-37 was found to be slimy, rod-shaped, positive towards catalase, mannitol, glycerol, urea, VP tests and fermentation of glucose, sucrose and lactose, whereas negative towards gram staining, spore formation, oxidase, amylase, citrate, methyl red and nitrate reduction tests. Antibiotic screening showed that the bacterium was sensitive to norfloxacin, gentamycin, chloramphenicol and ciprofloxacin whereas it was resistant

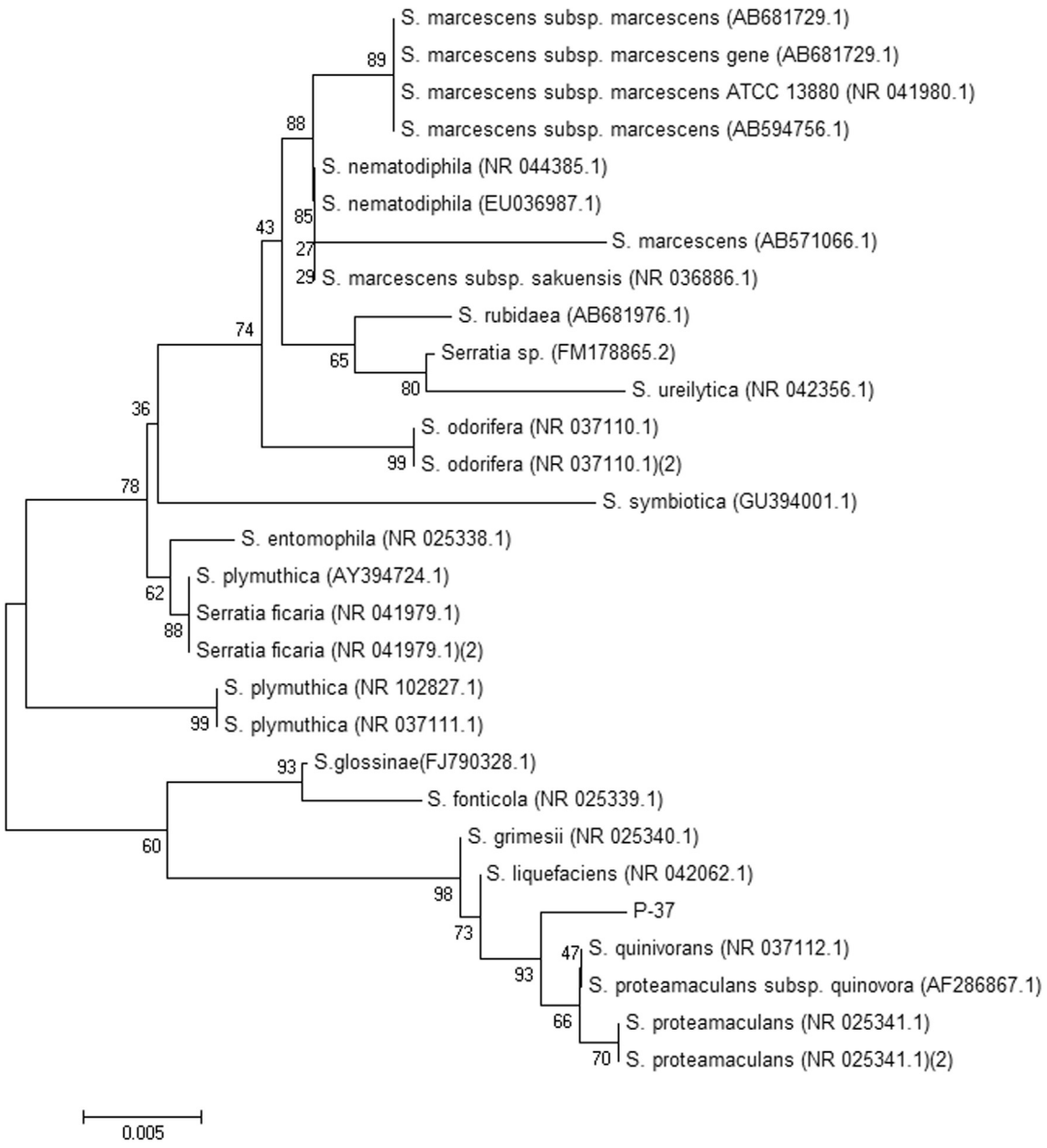

Figure 1 Phylogenetic tree based on 16S rRNA gene sequences by Neighbor Joining method (using MEGA 6.0), showing the relationship between strain PSB-26 and other members of the *Serratia* sp. The Genbank nucleotide accession numbers are listed next to the strain names. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The scale bars represent 0.005 substitution/site.

to ampicillin. Based on these morphological and biochemical characteristics, the bacterium was tentatively assigned to the genus *Serratia* sp. Further confirmation of genus *Serratia* was accomplished by BLAST analysis data of the 16S rRNA gene sequence, which showed 99% similarity with the genus *Serratia* sp. The gene bank accession number of the strain is KR632647. A phylogenetic tree was constructed by comparing the nucleotide sequences of 16S rRNA sequences of the isolate PSB-37 with different *Serratia* sp. from NCBI database. We found that our isolate is most closely related to *Serratia* sp. (Fig. 1).

3.2. Solubilisation of tricalcium phosphate

Evaluation of the phosphate solubilizing activity of the strain *Serratia* sp. (PSB-37) was carried out in NBRIP-broth medium at every 24 h, for a period of 264 h (data shown in Fig. 2). The phosphate solubilizing ability of the strain was increased from 0.0 μg/ml to 44.84 μg/ml as the pH decreased from 7.0 to 3.15. The pH of the medium decreased steadily after 6 h of incubation, but started to increase again after 48 h of incubation. Maximum phosphate solubilization was observed at 48 h (44.84 μg/ml, $p < 0.05$) of incubation with a maximum drop in the pH (3.15) of the medium (Fig. 2).

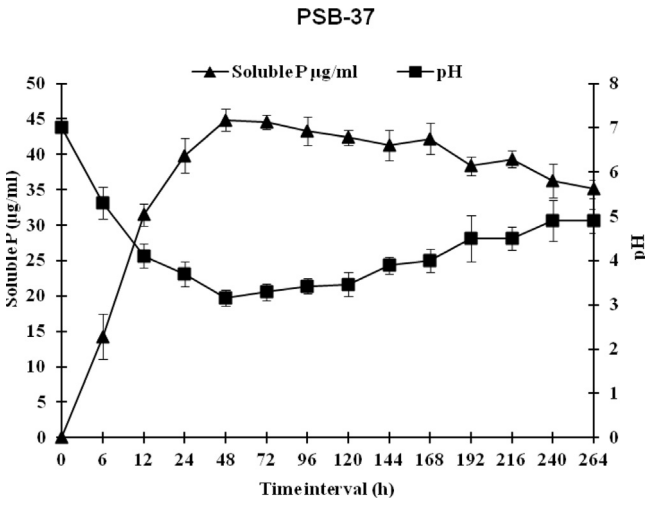

Figure 2 Time dependent tri-calcium phosphate solubilisation and drop of pH of the NBRIP broth medium by *Serratia* PSB-37 isolate.

3.3. Detection of organic acid

Detection of organic acids in the culture broth of the isolate, PSB-37, was determined through HPLC after 96 h of incubation. Three different organic acids, including lactic acid, malic acid and acetic acids were detected from the culture medium of the isolate PSB-37. These acids were confirmed by comparing HPLC results of six pure organic acids as standard (Fig. 3). Deionized water was taken as a control. Out of the three different organic acids, lactic acid was produced in the largest quantity (599.5 mg/l), followed by malic acid (237 mg/l) and acetic acid (5.0 mg/l).

3.4. Acid phosphatase production

Acid phosphatase production was carried out in NBRIP broth medium inoculated with the bacterial isolate, PSB-37. Significantly ($p < 0.05$) higher enzyme production (76.808 U/ml) was recorded at 48 h of incubation and decreased upon further incubation up to 192 h (data not shown). The NBRIP broth medium without inoculation did not exhibit any presence of the enzyme.

3.5. Optimization of acid phosphatase production

The effect of different pH, temperature, agitation rate, carbon sources and nitrogen sources were studied for optimum acid phosphatase production after 48 h of incubation. Temperature is one of the most critical parameters controlling any bioprocess. The optimum temperature for acid phosphatase production was investigated at 10 °C increments from 25 °C to 65 °C, maintaining the pH at 5.0 over 48 h of incubation. In shaken cultures, 45 °C was found to be the optimum temperature for acid phosphatase production (77.87 U/ml, $p < 0.0001$). Acid phosphatase production was found to decrease beyond the optimum temperature (Fig. 4a). The effects of agitation rate on the production of acid phosphatase were investigated at temperature 45 °C and after 48 h of incubation period. Fig. 4b showed that highest acid phosphatase production was obtained (80.40 U/ml, $p < 0.0001$) when agitated at 100 rpm. At this agitation speed an adequate amount of air was introduced into the medium to supply needed dissolved oxygen. The effects of pH on the production of acid phosphatase were investigated after 48 h of incubation period, at temperature 45 °C and agitation rate of 100 rpm. The effect of pH on enzyme production showed a gradual increase in enzyme production from pH 3.0 to 5.0, with maximum enzyme production at pH 5.0 (80.66 U/ml, $p < 0.0001$), and decreased

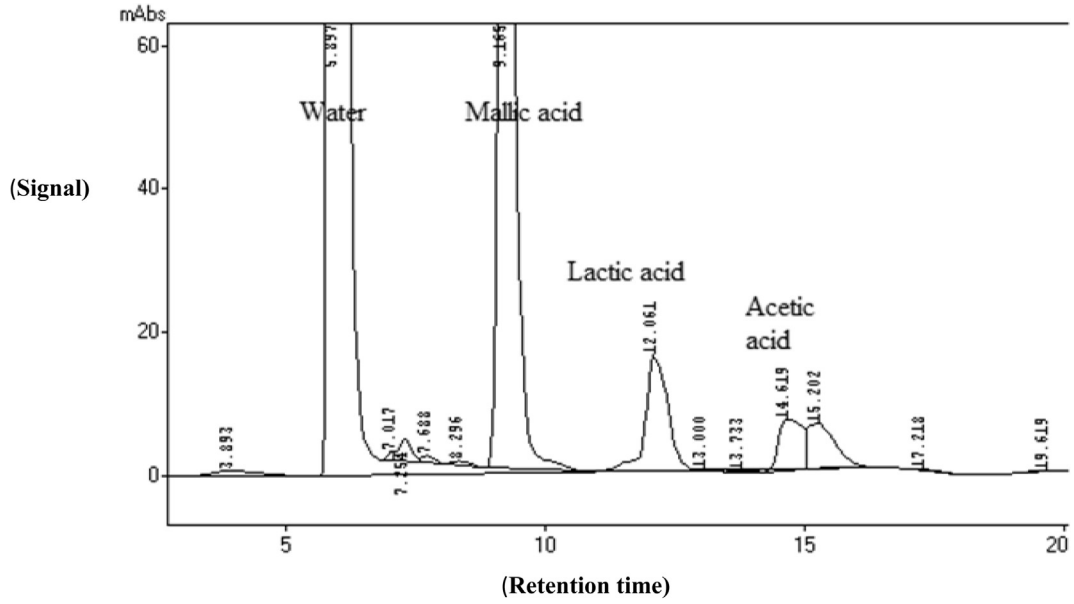

Figure 3 HPLC analysis of organic acids in the culture supernatant of isolates PSB-37 at 144 h of incubation in NBRIP broth. The corresponding peaks detected in culture medium were of lactic acid, malic acid and acetic acids including eight unknown peaks (3.8, 7.0, 7.6, 8.2, 13.0, 13.7, 17.2 and 19.6).

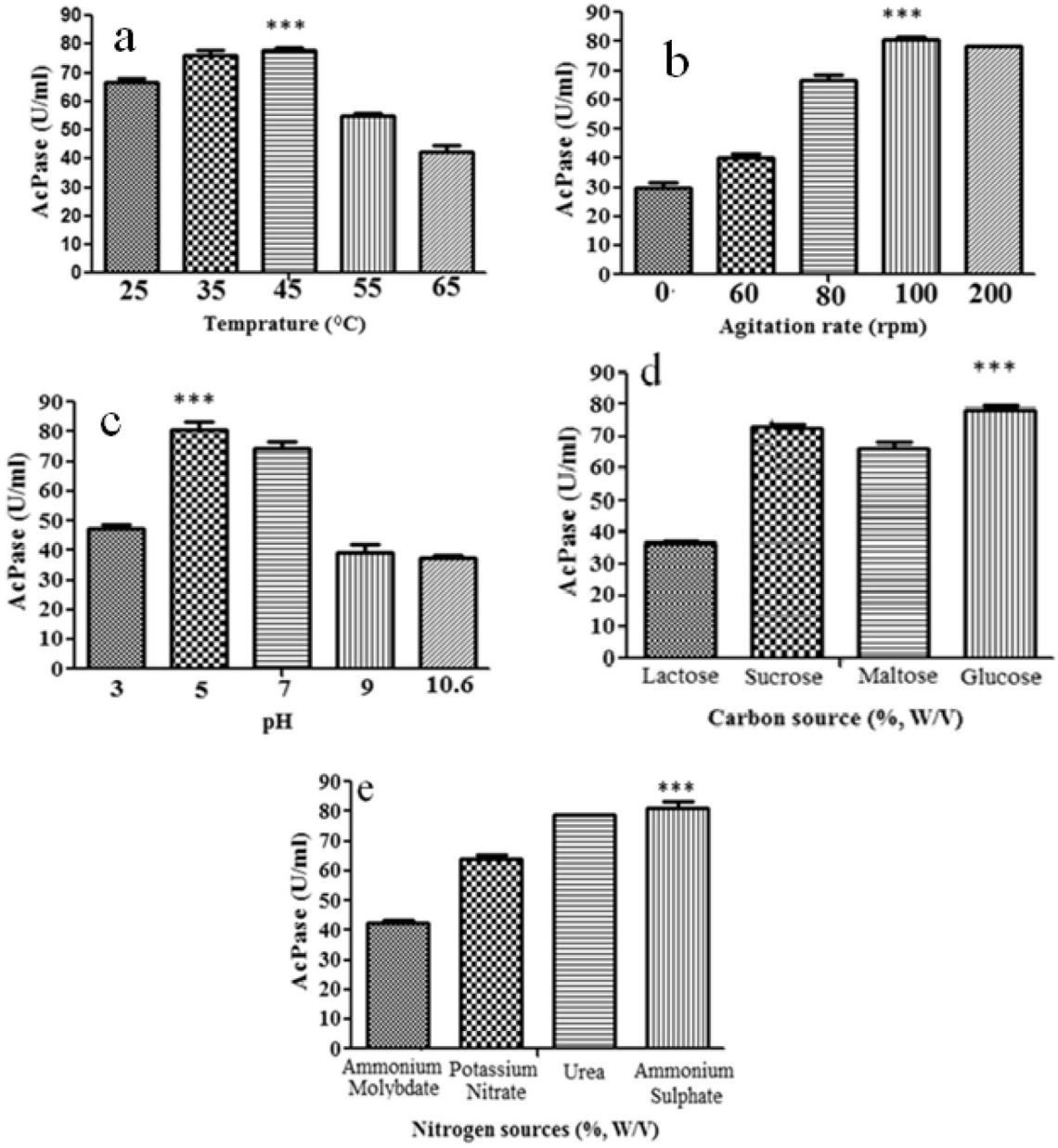

Figure 4 Effect of different growth parameters (a) Temperature, (b) Shaking velocity, (c) pH, (d) Carbon sources and (e) Nitrogen sources on crude acid phosphatase production by the isolate, PSB-37 (*** denotes *p*-value < 0.0001).

thereafter (Fig. 4c). Keeping the above optimized condition it was also observed that among the various carbon supplements used, the medium containing original carbon source as a glucose yielded maximum acid phosphatase production (80.66 U/ml, $p < 0.0001$), followed by sucrose and maltose with acid phosphatase values of 73 U/ml and 66.83 U/ml respectively. The lowest acid phosphatase production (36.33 U/ml) was observed in the presence of lactose (Fig. 4d). Keeping the incubation period of 48 h, temperature of 45 °C, agitation rate of 100 rpm, pH, 5.0 and glucose as a carbon source, when four different nitrogen sources were tested to optimize acid phosphatase production, it was observed that maximum production occurred (80.92 U/ml, $p < 0.0001$) with the original nitrogen source, ammonium sulphate when supplied to the medium, than other nitrogen sources such as Urea (78.83 U/ml) and potassium nitrate (64.16 U/ml) respectively. The lowest acid phosphatase production (42.16 U/ml) was observed when ammonium molybdate was used as a nitrogen source (Fig. 4e).

Hence under optimized sets of conditions (incubation period 48 h, initial pH of 5.0, a growth temperature of 45 °C, shaking velocity of 100 rpm, glucose as a carbon source and ammonium sulphate as a nitrogen source), the bacterial isolate, PSB-37, showed maximum phosphatase activity of 80.92 U/ml.

Table 1 Partial purification of crude acid phosphatase.

Isolates PSB-37	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 extract	50	12.0	600	76.8	3840	6.4	1.0	100%
70% $(NH_4)_2SO_4$ precipitation and dialysis	15	9.0	135	86.23	1293.45	9.58	1.5	36.68%

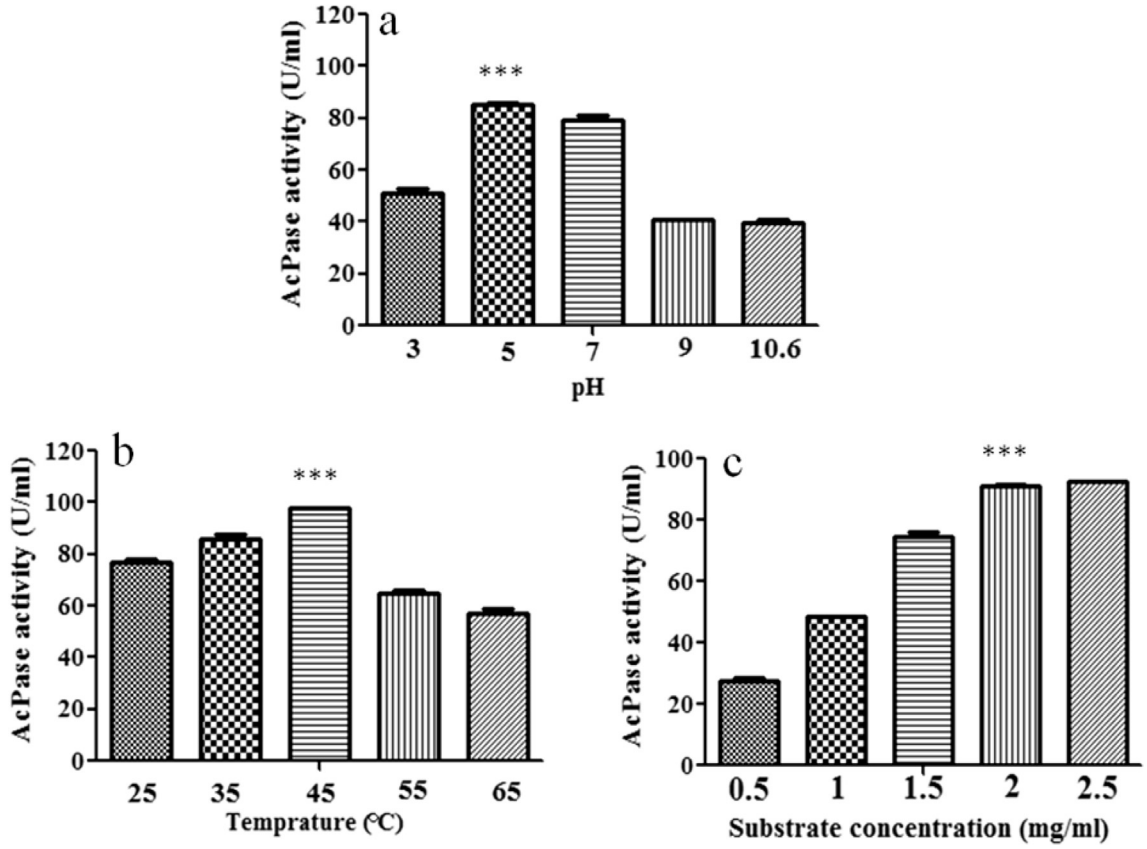

Figure 5 Effect of (a) different pH (b) different temperature and (c) different substrate concentration on partially purified acid phosphatase activity (*** denotes p-value < 0.0001).

3.6. Partial purification of acid phosphatase

Partially purified acid phosphatase from the bacterial isolate PSB-37, exhibited a protein concentration of 9 mg/ml, with a specific activity of 9.58 U/mg, which corresponds to 1.5 fold purification and 33.68% yield (Table 1).

3.7. Characterization of partially purified acid phosphatase

The partially purified acid phosphatase from *Serratia* sp. was characterized with different pH (3.0–10.6) temperature (25–65 °C) and substrate concentration (0.5–2.5 mg/ml). The effect of pH on partially purified phosphatase activity was determined by assaying the enzyme activity in buffers having different pH, ranged from pH 3.0 to 10.6. The maximum activity of acid phosphatase was recorded (85.6 U/ml, $p < 0.0001$) at pH 5.0 (Fig. 5a). Enzyme activity was also greatly influenced by the incubation temperature when tested over a wide range of temperatures. The optimum temperature for acid phosphatase activity was found to be 45 °C, with an enzyme activity of 97.87 U/ml ($p < 0.0001$). Temperatures higher than 45 °C resulted in a reduction in acid phosphatase activity probably due to the denaturation of the enzyme (Fig. 5b). The effect of acid phosphatase activity by the isolate PSB-37 was studied over a wide range (0.5–2.5 mg/ml) of substrate concentration. It has been observed that the enzyme activity increased with an increase in substrate concentration, with a maximum activity of 92.7 U/ml ($p < 0.0001$) at 2.5 mg/ml (Fig. 5c).

4. Discussion

Phosphate solubilizing bacteria forming a halo zone on NBRIP-agar medium were isolated from the mangrove soil of the Mahanadi river delta. However, the result of the halo

based technique is not always reliable. Many isolates which did not produce any visible halo zones on agar plate could also solubilize various types of insoluble inorganic phosphate in liquid medium [21]. This may be due to various diffusion rates of different organic acids secreted by an organism [34]. Therefore, phosphate solubilizing bacteria were further screened in NBRIP-BPB broth medium to evaluate their phosphate solubilizing efficiency. NBRIP medium contains bromophenol blue (a pH indicator dye), which changes its colour due to the decrease in pH of the medium. Hence, phosphate solubilizing efficiency of microorganisms can be easily screened based on visual observation [29].

Based on biochemical and partial 16S rRNA gene sequence analysis the bacterial isolate, PSB-37 shared 99% similarity with *Serratia* sp. The bacterial isolates, PSB-37, exhibited maximum phosphate solubilization of 44.85 μg/ml at 48 h of incubation, which corresponds to 44.85 mg/l. In comparison to the present study, phosphate solubilizations in different ranges were also reported from other mangrove ecosystems. Seven bacterial sp., such as two *Bacillus subtilis,* three *Pseudomonas* sp. and two *Azotobacter* sp., isolated from mangrove soil of Chollangi, East Godavari, exhibited a phosphate solubilizing ability in the range of 80–100 mg/l [6]. Pramod and Dhevendran [38] isolated IPSB *Vibrio* sp. and *Pseudomonas* sp. from Cochin mangrove of India, which could solubilize phosphate in the range of 0.5–0.55 mg/l. Kathiresan and Selvam [24] isolated 24 phosphate solubilizing bacteria from the mangrove soils of the Vellar estuary at Parangipettai, southeast coast of India, whose phosphate solubilizing efficiency was in the range of 0.012–0.141 mg/l. Much higher phosphate solubilizing activity (400 mg/l) was also reported by the bacterial population in an arid mangrove ecosystem in Mexico [52].

In the present study, the bacterial isolate, PSB-37, showed 3.15–4.0 units of decrease in pH of the medium which is similar to the findings of Perez et al. [35] who also observed 3.2–4.0 units decrease in pH of the medium during phosphate solubilization. The inverse relationship observed between pH and soluble-phosphate concentration indicates that organic acid production by the strain plays a significant role in the acidification of the medium, facilitating the phosphate solubilization. The rise in pH could be due to utilization of organic acid or the production of alkaline compounds [1]. A similar inverse relationship between pH and soluble phosphate has been previously reported [23].

HPLC analysis indicates the presence of malic acid, lactic acid, and acetic acid in the broth culture of PSB-37 (Fig. 3). Alam et al. [2] also reported the presence of several organic acids, such as citric acid, oxalic acid, acetic acid, gluconic acid, through HPLC in the culture broth of phosphate solubilizing microorganisms. Glucose is the principal element of NBRIP medium, and phosphate solubilizing microorganisms prefer glucose as a carbon source to produce organic acids [34]. Both in plants and microorganisms, the primary mechanisms of P solubilisation are H^+ excretion, organic acid production, and acid phosphatase biosynthesis [4]. 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 [22]. Organic acids may also increase P availability by blocking P absorption sites on soil particles or by forming complexes with cations on the soil mineral surface [8].

Serratia sp inhabiting soil express a significant level of acid phosphatase [42,44,46,14,17]. In the present study it has been observed that maximum acid phosphatase produced by *Serratia* sp. occurred (76.8 U/ml) after 48 h of incubation. A significant amount of acid phosphatase activity has also been reported from *Serratia* sp., as well as different bacterial genera by several researchers [18,39,53].

Phosphatase production is greatly influenced by growth parameters such as different pH, temperature, agitation velocity, carbon source and nitrogen source. In order to optimize the crude enzyme production, there is a need to evaluate optimum condition of these parameters under laboratory conditions.

The effect of temperature on enzyme activity is a critical parameter which usually varies from organism to organism [25]. The study conducted by Frankena et al. [16] showed that there was a link between enzyme synthesis and energy metabolism in bacteria and this was controlled by the temperature and oxygen uptake. As production of extra-cellular enzymes is influenced by temperature, their secretion is possibly influenced by changing the physical properties of the cell membrane [41]. In the present study, maximum acid phosphatase activity was observed at 45 °C. Acid phosphatase activity in the same range was also reported earlier [19].

Variation in agitation or shaking speed has been found to influence the extent of enzyme production [33]. Higher agitation velocity can increase the oxygen pressure of the system but can't bring about an increase in enzyme production because at higher agitation rates the structure of the enzyme would be altered [43]. Lowering the aeration rate can also cause a drastic reduction in the enzyme yields [54]. In the present study, optimal acid phosphatase production by the bacterial isolate, PSB-37, was observed at 100 rpm. Beyond the optimum agitation rate the enzyme production found to be decreased.

Increase or decrease in pH beyond the optimum value can affect the active site of the amino acids as the enzyme is unable to form an enzyme substrate complex and thus there is decrease in enzyme activity [45]. The bacterium under study was found to optimally synthesize acid phosphatase at pH-5.0. Malke [28] also reported acid phosphatase activity by *Streptococcus equisimilis* which exhibited optimum activity at pH 5.0. Acid phosphatase activity less than pH 5.0 was also reported earlier [36,55].

The use of low cost substrates for the production of industrial enzymes is one of the alternative ways to reduce production costs significantly. The amount of enzyme produced by each substrate differs depending on the type of carbon and nitrogen source preferred by organisms. Investigations on the impact of carbon and nitrogen supplements on enzyme production revealed that not all carbon and nitrogen sources would act as an enhancer for the production of enzymes. In the present finding, the bacterial isolate, PSB-37, showed maximum acid phosphatase production in medium supplemented with glucose as a carbon source and ammonium sulphate as a nitrogen source.

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. The purification process may separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins. In the present study, the crude

enzyme solution was partially purified up to 1.5 fold with a yield and specific activity of 33.68% and 9.58 U/mg respectively. Poirier and Holt [37] partially purified acid phosphatase from *Capnocytophaga ochracea* and recorded a 50% yield. Gonzalez et al. [19] in their experiments on phosphatases from *Myxococcus coralloides D* obtained 47.4% yield and specific activity of 137.7 U/mg for acid phosphatase.

Enzyme purification is vital for the characterization of the function, structure and interactions of the protein of interest. Hence partially purified acid phosphatase from the isolate, PSB-37, was characterized with different parameters such as pH, temperature and substrate concentrations. After partial purification, maximum acid phosphatase activity by the isolate PSB-37 was observed at pH 5.0. Similar reports of acid phosphatase production were also reported earlier [47,5]. Acid phosphatase activity higher than pH-5.0 was also reported [30,32]. Increase in temperature accelerates the velocity of an enzyme catalyzed reaction until an optimum level is reached after which the velocity decreases and finally results in the denaturation of the enzyme. The effect of temperature on enzyme activity also varies from organism to organism. It has been observed that after partial purification, the highest acid phosphatase activity obtained at 45 °C and decreased thereafter. Acid phosphatase activity lower or higher than the observed temperature was also reported earlier [30]. The effect of different pNPP (substrate) concentrations (0.5–2.5 mg/ml) on acid phosphatase activity produced by the isolate revealed that maximum acid phosphatase activity increased with increasing substrate concentration, and optimum enzyme activity was obtained at 2.5 mg/ml. Further the activity was found to be constant. The constant enzyme activity at higher substrate concentration may be due to the saturation of the binding sites of the enzyme with the substrate [51].

5. Conclusion

Serratia sp a gram negative bacterium, is an opportunistic human pathogen causing a variety of diseases such as bacterial meningitis, brain abscess in new born and nosocomial infection due to multidrug resistance in some species. Although the *Serratia* strain is an opportunistic pathogen, it has been reported to promote plant growth by inducing resistance against plant pathogens. This study provides evidence for the existence of agriculturally important bacteria in the relatively unexplored mangrove environment, which may help taxonomists, enzymologists, and even some agriculturalists in their own research. Moreover, due to the phosphate solubilizing and acid phosphatase production ability of the bacterium, it may have probable use as bio-inoculants to increase soil fertility by minimizing fertilizer application, which can promote sustainable agriculture and help to meet future needs.

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