

Study of Plant Growth Promoting Effects by a Phosphate Solubilizing *Acinetobacter sp* Strain AM1(OR430119.1) on *Vigna radiata*

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Abstract

In agricultural soils, soil salinity and low phosphorus availability due to frequent precipitation of phosphorus into insoluble complexes lead to unsustainable reliance on chemical fertilizers to grow crops. This degrades soil quality over time and thus presents significant challenges to agriculture. This study deals with the isolation and characterization of a salt-tolerant, phosphate-solubilizing bacterium from the naturally saline soil in Canning, West Bengal, India, to evaluate its potential as an eco-friendly biofertilizer for stress-affected crops in such adverse soils. This study aims to isolate and characterize such high efficiency PSB from soil that can serve as a biofertilizer. Taxonomic identification via phenotypic, and 16S rRNA sequencing classified the isolate as *Acinetobacter sp. strain AM1* (NCBI Accession: OR430119.1). Quantitative analysis revealed that the bacterium is a phosphate solubilizer. Soluble phosphate concentrations in the culture medium increased significantly from $5.02 \pm 0.9 \mu\text{g/ml}$ on day 1 to $42.37 \pm 0.11 \mu\text{g/ml}$ by day 5 post-inoculation. The strain also exhibited tolerance to salinity (up to 3% NaCl), reducing residual sodium ions in the medium from 10.015 g/l to 9.350 g/l through cellular uptake. Physiological profiling demonstrated that the bacterium also synthesizes 0.16 mg/ml of the indole acetic acid (IAA) — a phytohormone that helps in plant growth. To evaluate plant growth promoting capacity, a 25-day pot experiment was conducted with green gram (*Vigna radiata*) seeds using sawdust, cow dung, and combined matrices as bacterial carriers. Furthermore, bio-inoculated soil enhanced chemical fertility, increasing soil nitrogen by 9.51%, potassium by 12.52%, and phosphorus by 25% relative to the uninoculated control. This study, thus by statistical validation of improved plant biomass and soil macronutrient profiles, proves that *Acinetobacter sp. strain AM1* has clear potential as a functional biofertilizer for sustainable agriculture in saline, soluble phosphate-poor soil under standard and saline (3% NaCl) soil conditions.



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Abbreviation

PGP	(Plant growth promoting),
NPK	(Nitrogen, phosphorous, Potassium),
PSB	(Phosphate solubilising Bacteria),
IAA	(Indole acetic acid)

Introduction

Phosphorus (P) is the second only to nitrogen among mineral nutrients which required for plant growth and development and involved in important metabolic pathways like nutrient uptake, biological oxidation, and energy metabolism.¹⁻³ In soil, it precipitates as orthophosphate. Tremendous use of inorganic fertilizers which can provide a source of phosphorous to the plant can lead to deterioration of quality of soil with less availability of nutrients to the crops.⁴ Phosphorus solubilizing bacteria (PSB) releases inorganic and organic soil P pools by solubilization and mineralization and enhances Phosphorus availability to plants, thus play an important role in Phosphorus nutrition.⁵ Use of phosphorus solubilizing bacteria (PSB) as inoculants increases P uptake. These bacteria also increase the possibility of using phosphatic rocks in crop production.⁶ The salt-tolerant or halophilic soil microorganisms that possess the ability to solubilize insoluble phosphorus also facilitate the development of saline-alkali soil-based agriculture.⁷ Therefore, for improving plant phosphorus uptake inoculation of soil or crops with phosphate solubilizing/mineralizing microorganisms is a promising.⁸ Additionally, phosphate solubilising bacteria can enhance the number of trace elements, soil nitrogen, potassium content and secrete a variety of plant growth-promoting hormones which is essential to increase plant growth.⁹

Since the 1950s Phosphate solubilizing bacteria (PSB) have been used as bio fertilizers. PSB secretes organic acids like carboxylic acid, which dissolves inorganic phosphates.^{8,10,11} To decrease the pH and to combine PO_4^{3-} to form HPO_4^{2-} or H_2PO_4^- these organic acids ionize protons and increase phosphate solubility. Organic acid anions are also able to form a complex with metal cations (Ca^{2+} , Al^{3+} , and Fe^{3+}) and consequently, release PO_4^{3-} . Similarly, which has been reported by several studies that PSB-assisted phytoextraction may improve the mobility of Cadmium in soil.¹²⁻¹⁴ Use of these microorganisms

as eco-friendly bio fertilizer helps to reduce the use of phosphate fertilizers, which is expensive as well.

However, despite the developments made in biofertilizer research, there is one crucial area where further research is still needed: a lack of strains capable of tolerating multiple forms of environmental stress. Specifically, what's required is a strain that can effectively act as biofertilizers which is able to carry out phosphate solubilization while serving as IAA producers, salt tolerance strain, heavy metal resistant, and antibiotics resistant strain. The need for such versatile strains is particularly urgent in environmentally fragile areas like the Sundarbans. Due to tsunami and several natural disasters, soil of this site is saline. Additionally, as a result of the rapid transformation of the Sundarbans area into a major tourist attraction, there have been many motorized boats on its waters. Constant exposure to commercial paint from these motorized boats has led to excessive amounts of lead (Pb) and other heavy metals in the soils. Oil spillage and other hydrocarbon leakage decrease the fertility of soil.⁴ These soils form highly stressful ecosystems, characterized by both heavy metals and antibiotics, thus making it an area of co-selection for both heavy metals and antibiotics. The major aim of this research will thus involve isolation, screening, and evaluation of a new generation of multipurpose biofertilizers with combined heavy metal and multidrug resistance properties.

Materials and Methods

Isolation and Screening

Soil samples were collected from a selected site (22.3209° N, 88.6724° E) in Canning, South 24 Parganas, West Bengal, India. Physiochemical properties of the soil were measured like pH, Salinity etc. Sterile collection equipment (spatula, test tube) was used. A series of 6 serial dilutions were carried out using 1g of soil in 9ml sterile water as stock. 0.1ml aliquot from dilutions 10^{-5} and 10^{-6}

were plated on Pikovskaya agar media (readymade supplied by Himedia; composition in g/l: yeast extract 0.50g, dextrose 10.00g, calcium phosphate 5.0g, ammonium sulphate 0.50g, potassium chloride 0.20g, magnesium sulphate 0.10g, manganese sulphate 0.0001g, ferrous sulphate 0.0001g, agar 2%). The inoculated plates were incubated at 37°C for 72 hours. The colonies capable of phosphate solubilization were identified by presence of clear halo zones around them. These colonies were picked and via streak plate method pure cultures were obtained. The pure colonies were spotted on separate Pikovskaya agar plates and incubated for 24 hours at 37°C and their solubilization index (SI)¹⁵ were calculated as per equation.

$$\text{Solubilization index (SI)} = \frac{(\text{Diameter of colony} + \text{Halo zone})}{\text{Diameter of colony}}$$

The colony having the maximum SI value was selected for further study.

Phylogeny

To isolate bacterial genomic DNA from the overnight culture grown in Luria-Bertani broth HiMedia's HiPurA™ Bacterial Genomic DNA Purification was used. Then this DNA was used for 16S rRNA amplification. Here, two universal primers, (27F 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGACTT-3') were used to amplify 16S rRNA of the bacterium.^{16,17} A 25 µL of total volume was taken for PCR amplification with 2.5 µL buffer containing MgCl₂, 2 µL template DNA, 0.5 µL each of the forward and reverse primers, 0.5 µL dNTP mix, 0.25 µL Taq DNA polymerase and 18.75 µL nuclease free water. Next, the PCR was performed parameters were and the 50°C for 2 min, 95°C for 10 min, and then 38 cycles at 95°C for 30 s and 62°C for 1 min. After purification of 16S rDNA by gel electrophoresis, sequencing of amplicon was done using Applied Biosystem 3500 series genetic analyser system. The obtained 16S DNA sequence was then used to determine the most closely related sequence available in GenBank database using NCBI BLAST. Phylogenetic tree was constructed using the MEGA 11 software.

Characterization

The morphological and biochemical properties of the isolate were determined as a part of the study by the classical morphological and biochemical

tests. The isolate *Acinetobacter* sp. strain AM1, was routinely grown on Pikovskaya agar media (readymade supplied by Hi-media laboratory). For morphological characterization, the colony morphology such as, shape, size, colour, texture and margin were noted after incubation for 72 hours at 37°C. Gram staining and capsule staining were performed by the standard protocols and the cells were visualised under 1000X magnification and 400X magnification respectively.¹⁸⁻²⁰ Motility test was performed by the standard hanging drop method²¹ and visualisation was done under 45X objective lens of the microscope (Olympus Magnus MLX-B).

To discern biochemical characteristics, variety of tests were performed. Catalase activity was evaluated using previously determined method using 3% H₂O₂.²² Amylase activity was determined by the standard starch hydrolysis test using iodine as the reagent.²² Urease and Nitrogenase tests were carried by growing the isolate on Urea agar media (observation of colour change) and Burk's agar media (observation of growth in nitrogen free media) respectively. Presumptive and IMViC tests were carried out according to standard protocol.²³⁻²⁵ Further, the several experiments were carried out in triplicates in order to characterize them. Susceptibility to the antibiotics – Streptomycin (25), Chloramphenicol (30) and Tetracycline (30) was tested by the disc diffusion procedure (µg/disc) on LB (Luria Bertani agar from Himedia laboratory) media for 24 hours at 37°C using antibiotic discs obtained from Himedia laboratory. Tolerance against heavy metals – Cu, Hg, Zn and Pb was tested by cup plate method on LB agar media for 48 hours at 37°C for up-to 30 µg/ml concentration of the respective heavy metals. Sensitivity to salt was tested using LB media supplemented with varying percentages of NaCl concentrations (1% to 15%). Salt uptake ability of the isolate was tested by growing the bacteria in LB broth supplemented with 3% NaCl for 24 hours at 37°C after which supernatant was collected by centrifugation (Spinwin MC-01 microcentrifuge) at 4000rpm for 10 minutes. The amount of residual Na ions presents in the broth before and after bacterial growth was measured by flame spectroscopy (Systronics µ Controller Based Flame photometer with Compressor - Type 128; LPG gas as fuel for the Flame; 3 ml sample required for test). The aero tolerance nature of the isolate was determined by the growth position in test tube containing nutrient

broth media after 24 hours of incubation at 37°C. Growth characteristics on a variety of carbon sources (Starch, Glucose, Fructose, Sucrose, Lactose and Maltose) and nitrogen sources (Alanine, asparagine, aspartic acid, gelatine and ammonium dihydrogen phosphate) were evaluated to check the best suitable nutrient sources for growth. OD (at 600nm) was noted using a spectrophotometer (Schimadzu - UV 1800) to measure the turbidity after 24 hours of incubation at 37°C to determine the nutrient sources supporting maximum growth.

Growth Profile

The growth analysis was done in triplicate. 1% inoculum from a 48 hours culture was added to LB broth and growth was monitored at regular intervals (every 30 minutes) for 5 hours by measuring the OD (at 600nm) using a spectrophotometer (Schimadzu - UV 1800). An Absorbance vs Time graph was plotted. Also, at every 30 min interval, some of the culture was taken out, serially diluted and then plated followed by incubation at 37°C for 24 hours to obtain the CFU/ml for every 30 minutes interval.

Generation time was calculated from the CFU/ml of bacteria at the starting and the end of the log phase (corresponding to initial population and final population) as observed from the growth curve. Formula used was:

$$\text{Generation time} = \frac{\text{Time interval}}{3.3 * \log \left(\frac{\text{Final population}}{\text{Initial population}} \right)}$$

Determination of Organic Acid and IAA Production

The ability of the isolate to produce organic acid was determined by using Bromothymol blue as an indicator. The isolate was grown for 48 hours at 37°C on Pikovskaya agar plate with added Bromothymol blue pH indicator.

Indole Acetic Acid production by the isolate was determined by colorimetric method using Salkowski reagent as an indicator.²⁶ To 2 ml bacterial supernatant, 4 ml Salkowski reagent was added followed by incubation for 90 minutes. Observation was made for colour change and O.D (at 530nm)

was measured. The obtained value was compared against pure IAA standard curve to determine the concentration of IAA produced.

Quantitative Estimation of Phosphate Solubilization

Phosphate solubilization by the bacterial isolate was assayed for 5 days post inoculation. In a 25 mL of Pikovskaya broth 0.1 mL of bacterial culture was inoculated and incubated at 37°C for 5 days. 1.5 mL of aliquots was withdrawn from the culture on each and every day up-to 5 days post inoculation and centrifuged for 8-10 mins at 10,000 rpm at room temperature to obtain the supernatants. The concentration of soluble phosphate was estimated by adding 4 mL of Phosphate reagent containing 2.5% ammonium molybdate, 6(N) H₂SO₄ and 10% ascorbic acid to 1 mL of the supernatant of each day and measuring the absorbance at 829 nm in a Shimadzu UV-1800 UV-Visible Spectrophotometer. Phosphate concentrations were calculated from a standard curve set up with different concentrations of sodium dihydrogen phosphate solution using the Phosphate reagent in a similar manner.

Determination of Plant Growth Promoting Activity of the Strain on Green Gram Plant

As AM1 showed various plant growth promoting ability it was introduced in soil with several carrier like sawdust, cowdung and their combination in a ratio of 2:1 that is 40gm carrier with 20ml of bacterial culture (CFU=2 X10⁸/ml) to check its ability of plant growth promotion. Germinated green gram seeds were sown on pots with carrier soil and control soil pot. In between 5 days interval for 25 days plant height was measured along with length of leaves. In another set of experiment Germinated Green gram plant were planted in soil containing 3 % NaCl in soil with sawdust as carrier containing bacterial inoculum (CFU=2 X10⁸/ml) and without inoculum to test whether this strain is capable of increasing plant growth in terms of both height of plant and leaf length measurement in soil containing stress factor like NaCl.

Also, NPK content of the soil was measured for evaluation of NPK content of soil before and after (25 days) bacterial inoculation.

Statistical Test

All the experiments were done in triplicates and subsequent standard errors were calculated using the formula $SE = \sigma / \sqrt{n}$, where SE is standard error, σ is the sample standard deviation and n is the sample size. ANOVA analysis were done for plant height and leaf length study at 5 % level of significance. All studies were conducted using MS-Excel.

Results

Isolation and Screening

Physiochemical properties of soil were measured. The soil collected was silty clay type with yellowish brown colour, pH- acidic (4.1), organic carbon -1.26%. Initially there were 6 morphologically different colonies showed halo zone around the colony in Pikovskaya Agar plates. Of the various different colonies obtained on the initial dilution plates, 3 showed halo zone around them and were

good phosphate solubilizers — AM1, AM2 and AM3. The solubilisation index varied significantly among the isolates for the same time and conditions of incubation. Of all three isolates, AM1 showed the highest solubilization index (0.8 ± 0.08) and was selected for further study.

Phylogeny

After amplification of genomic DNA by PCR, it was amplified using two universal primer and after NCBI BLAST analysis it can be concluded that AM1 showed highest similarity (92.61%) with *Acinetobacter oleivorans* strain SD12. The result obtained by morphological and other biochemical tests also confirms this bacterium is of *Acinetobacter* genus. The top few matches were used to construct the phylogenetic tree using MEGA 11 software (Fig 1). NCBI Accession number is given.

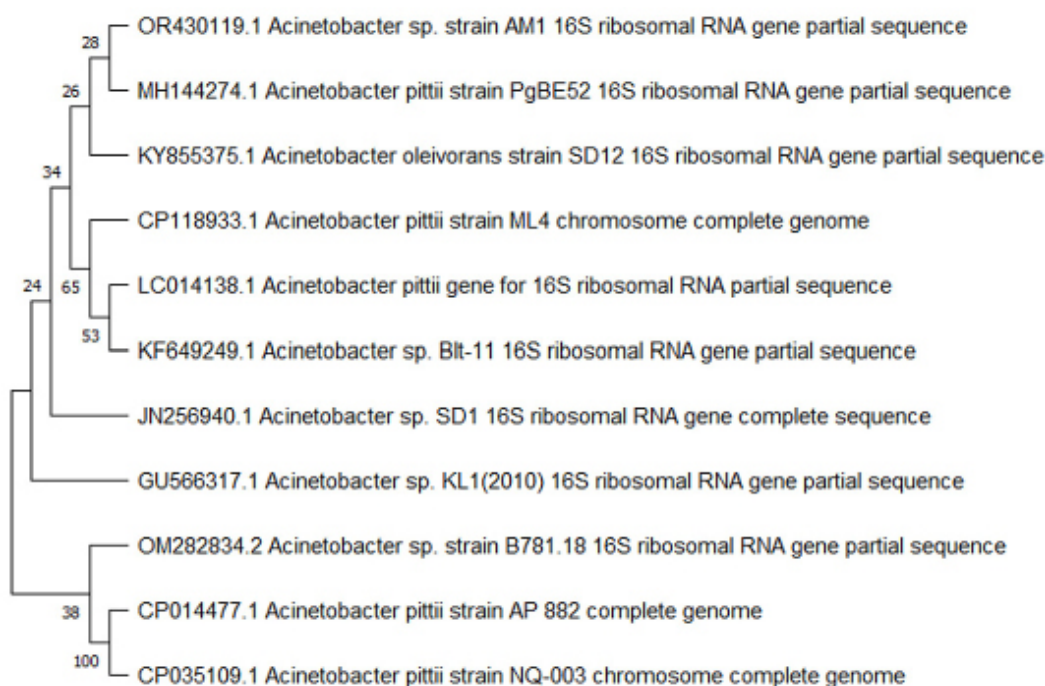


Fig.1: Phylogenetic tree for *Acinetobacter* sp. strain AM1 (OR430119.1).

Morphology and Biochemistry

The colonies of the isolate *Acinetobacter* sp. strain AM1 on Pikovskaya agar plates were observed to be circular in shape, white and opaque in colour and

medium in size with an entire margin and smooth texture. Gram-stained bacterial cells when viewed under 100X objective lens of the microscope were found to be cocci and occurred singly, or in clusters.

The isolate was Gram negative, showed absence of capsule and was motile as inferred from the clear erratic movement seen under 450X magnification.

Results obtained for the various biochemical tests are noted in the Table 1. Growth was observed at the top of the tube when inoculated in Nutrient broth for 24 hours at 37°C in standing condition indicating, this bacterium is aerobe. The OD obtained after incubation in various nitrogen and carbon sources showed that the highest growth was detected with

asparagine and starch as their preferred nutrient source. The comparative data is represented in the Fig2 and Fig3. The isolate was tolerant to salt till upto 3% and showed significant growth in 3% NaCl. The Na ion concentration in the supernatant obtained after 24 hours of incubation — 9.350 g/l — was found to be lower than that obtained before inoculation (initial Na ion concentration) — 10.015 g/l — which goes on to show that the isolate did uptake some amount of salt thus causing the decrease in the salt concentration in media.

Table 1: Biochemical tests (+ indicates positive test result; while – indicates negative test results)

Biochemical Tests	Results
Starch hydrolysis test	+
Catalase test	+
Urease test	-
Nitrogenase test	+
Presumptive test	+
Indole test	-
Methyl red test	+
Voges proskauer test	+
Citrate test	+
Susceptibility to Streptomycin (25µg/disc)	+
Suseptibility to Chloramphenicol (30µg/disc)	+
Susceptibility to tetracycline (30µg/disc)	+
Tolerance to heavy metals (Cu, Hg, Zn and Pb; upto 30µg/ml)	+

(All experiments were performed in triplicates)

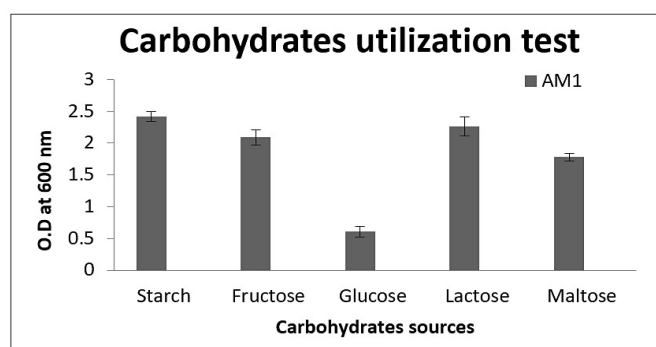


Fig.2: Utilization of different carbon sources.

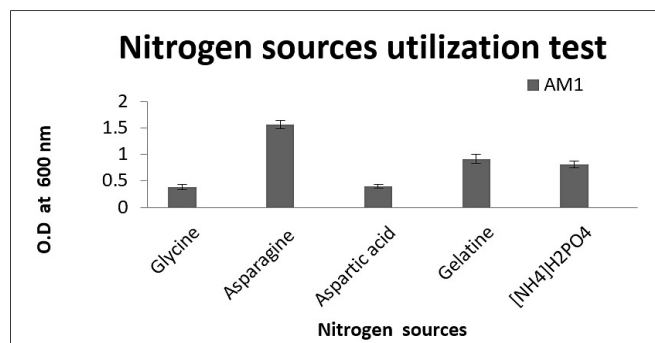


Fig.3: Utilization of different nitrogen sources.

Growth Profile Analysis

Growth profile analysis is important in bacteria to be used for bioremediation or bio inoculation. To overcome and healthily compete with the already existing flora

of the new environment, a short generation time is ideal. The growth curve obtained for the isolate is demonstrated in Fig.4. Generation time of the isolate was found to be around 57 minutes.

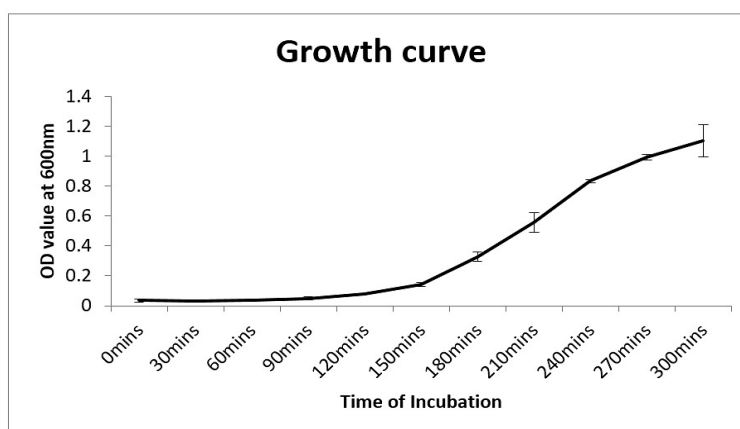


Fig.4: Determination of bacterial growth curve.

Determination of Organic Acid and IAA Production

Yellow colouration of the bromothymol blue supplemented Pikovskaya media was observed on the growth of the isolate indicating a decrease in the pH which is caused due to production of organic acids by the isolate. The isolate is positive for organic acid production.

The analysis for IAA production showed positive results indicating that the isolate was able to produce this phytohormone. The quantity of IAA produced, as determined from the IAA standard curve (Fig.5) was found to be 0.16mg/ml.

Estimation of Soluble Phosphate Concentration

To determine the rate of phosphate solubilisation by the isolate, soluble phosphate was estimated from culture supernatant each and every day up to 5 days post inoculation. A standard curve of sodium phosphate was constructed for phosphate estimation. For *Acinetobacter* sp. Strain AM1 strain soluble phosphate increases from 5.02 ± 0.9 microgram/ml at day 1 post inoculation to 42.37 ± 0.11 microgram/ml at 5 days post inoculation (Fig 6). This result also considered the fact that the organism is able to release some organic acid in the media that may help to convert insoluble phosphate to soluble one.

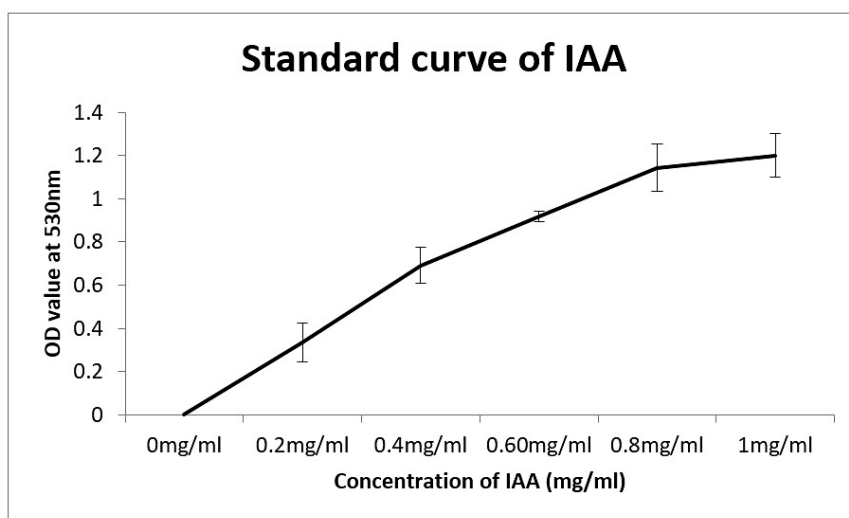


Fig.5: Standard curve of Indole Acetic Acid.

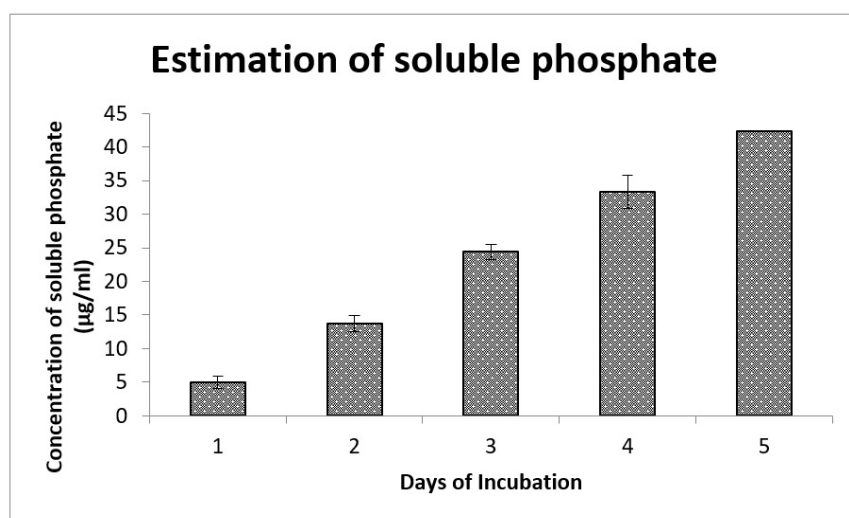


Fig 6: Soluble phosphate concentration in µg/ml produced in broth culture upto 5 days post inoculation due to inorganic phosphate solubilisation by *Acinetobacter* sp. Strain AM1.

Study of Plant Growth Promoting Effects on Green Gram Plant

The plant height and leaf sizes were measured at every 5 days interval for 25 days and graph for plant height and leaf sizes was drawn with these values. These two graphs indicate that both plant height (Fig.6) and leaf sizes (Fig.7) was maximum in which AM1 was inoculated with Sawdust as carrier. Fig 8 and Fig 9 showed variation of plant height and leaf length in presence of 3% NaCl treated soil. Again, both plant height and leaf length were increased

to bio-inoculated plant compared to that of control plant indicating this bacterium can efficiently promote plant growth both in normal soil and in saline soil. This, increase was found to be statistically not significant for all experimental sets (ANOVA analysis at 0.05 level of significance). Therefore, dry weight of the control plant and bio-inoculated plants were measured and ANOVA analysis at 5% level of significance were carried out. Dry weight of green gram plants was observed to increase after 25 days post-application of biofertilizers compared to control

plants as a result of incorporation of nutrients in the plants (increased growth of plants). Upon ANOVA analysis at 5% level of significance, the calculated $P < 0.05$ indicating that increase in dry weight of Green gram plants observed is significant.

Also, after 25 days nitrogen, potassium and phosphorus content of the soil increase about 9.51%, 12.52% and 25% respectively in respect to control soil (without carrier and AM1) (Table 2).

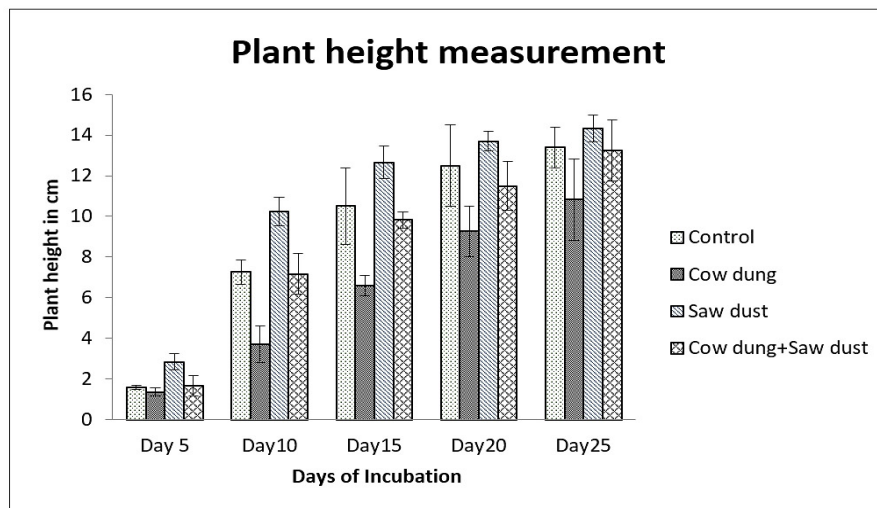


Fig.7: Determination of height of plant with or without bio-inoculum with different types of carriers.

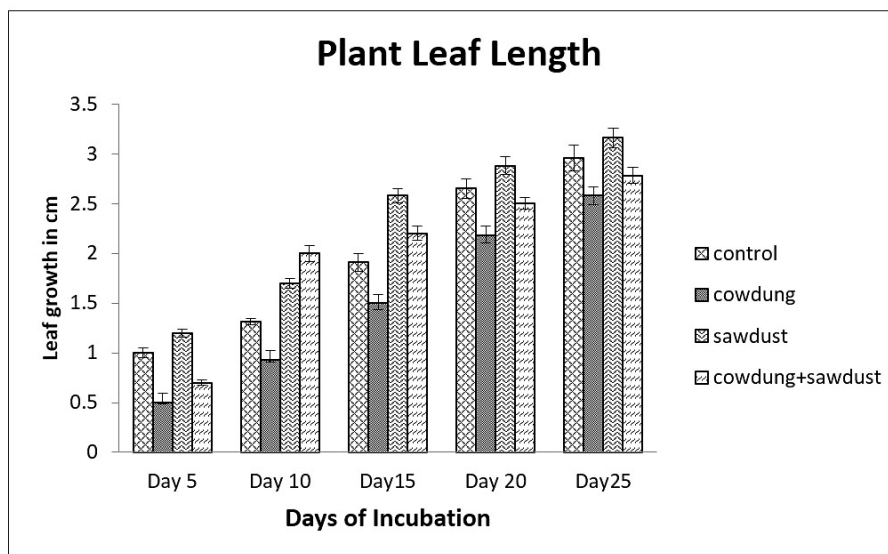


Fig.8: Determination of leaf length of plant with or without bio-inoculum with different types of carriers.

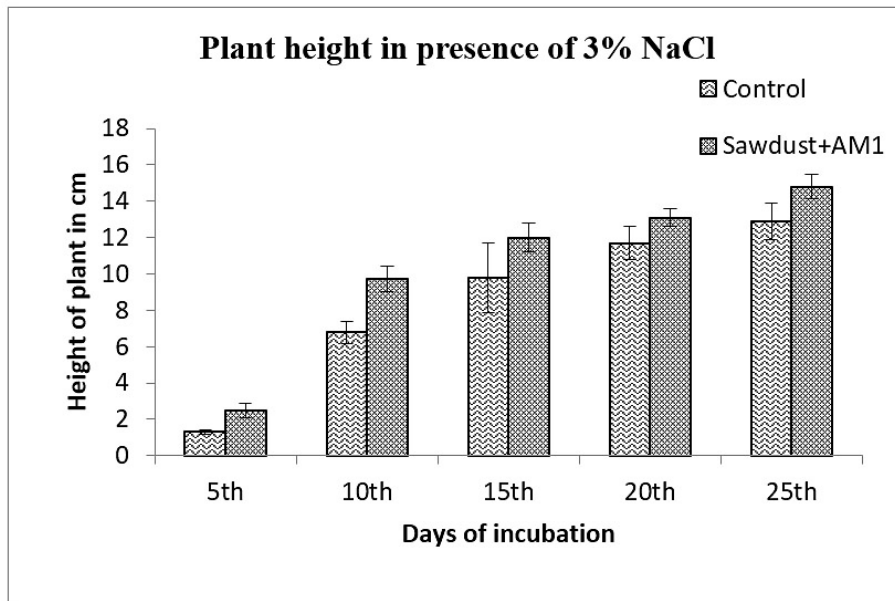


Fig 9: Determination of height of green gram plant in presence of 3 % NaCl with or without bio-inoculum.

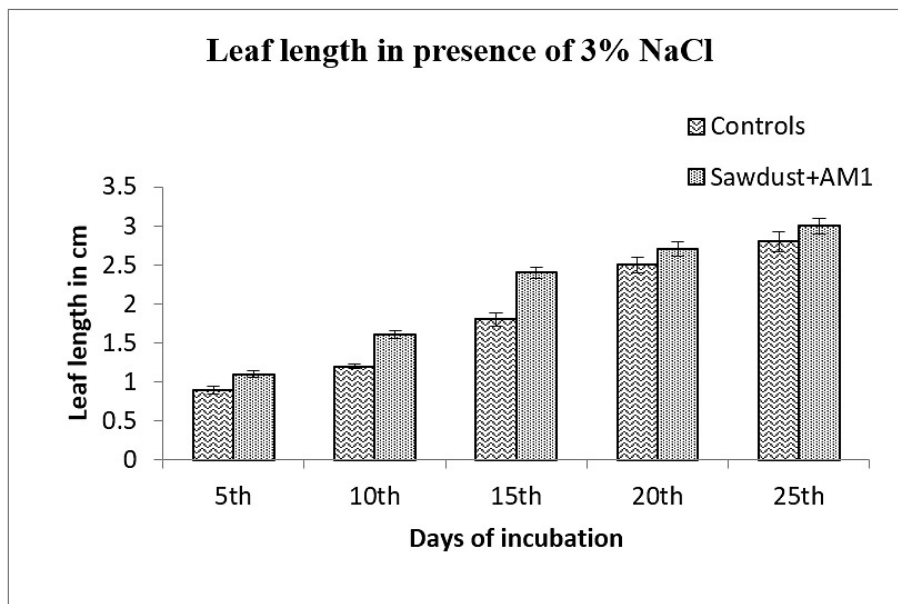


Fig 10: Determination of leaf length of green gram plant in presence of 3 % NaCl with or without bio-inoculum.

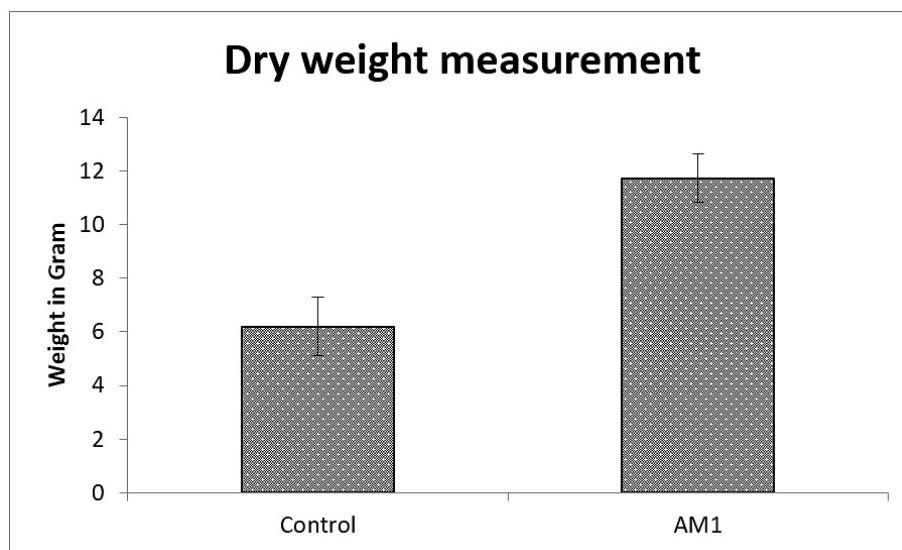


Fig 11: Measurement of dry weight of Control plant and AM1 Treated plant after 25 days of incubation.

Table 2: Table for ANOVA analysis at 5% level of significance

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	45.78844	1	45.78844	45.19461	0.00255	7.708647
Within Groups	4.052557	4	1.013139			
Total	49.84099	5				

Table 2: Determination of soil nitrogen, phosphorous and potassium content-

Sample	pH	N₂(kg/ha)	P₂O₅(kg/ha)	K₂O(kg/ha)
Control soil after 25 days	7.48	449.03	9.11	568.51
Bio-inoculated soil after 25 days	7.51	491.77	11.39	639.74

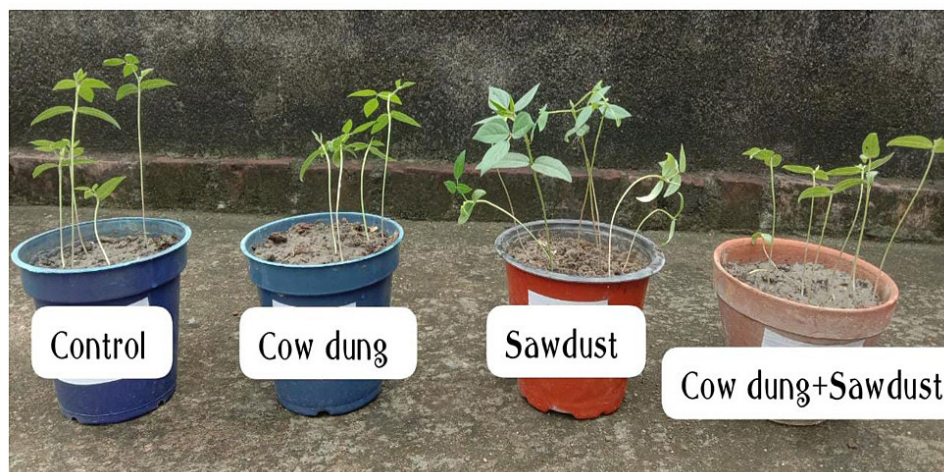


Fig.12: Green gram plants during the growth period.

Discussion

In this study the isolation of *Acinetobacter* sp. AM1 (NCBI Accession: OR430119.1) from the saline soils of Canning (22.3209° N, 88.6724° E), South 24 Parganas, West Bengal, India, makes this organism a promising candidate as a stress-tolerant biofertilizer for ecologically sensitive areas such as the Sundarbans. This bacterium is shown to have the ability of nutrient mobilization (insoluble phosphate to soluble phosphate) and phytohormone production (IAA) along with high salinity tolerance.

The most prominent function of AM1 as an agent of promoting plant growth is through its high efficiency of phosphate solubilization. Quantitative in vitro studies revealed that there was a significant increase in the concentration of soluble phosphate, from 5.02 ± 0.9 $\mu\text{g/ml}$ on day 1 to 42.37 ± 0.11 $\mu\text{g/ml}$ by day 5 post-inoculation. Solubilization of tricalcium phosphate to more soluble phosphate (bioavailable form) is caused by organic acid secretion from the bacteria, indicated by the change in colour in bromothymol blue-enriched Pikovskaya agar medium. The released protons from these organic acids reduce the pH, which chelates with metal cations such as Ca^{2+} , Al^{3+} , Fe^{3+} .

Other than nutrient mobilization, AM1 synthesizes 0.16 mg/ml of IAA. Being a critical phytohormone, the production of IAA by AM1 aids in promoting root

elongation and lateral growth, thus increasing the root surface area for increased uptake of nutrients.

The fragile ecosystem of Sundarban (canning) is subjected to environmental stressors in the form of saline water and heavy metals. The isolate AM1 was found to be highly adaptable to heavy metals (Cu, Hg, Zn, and Pb) up to $30\mu\text{g/ml}$, and exhibited resistance to commonly used antibiotics. Moreover, it has been observed through flame spectroscopy that this strain also exhibited tolerance to salinity (up to 3% NaCl), reducing residual sodium ions in the medium from 10.015 g/l to 9.350 g/l through cellular uptake.

Finally, the efficiency of AM1 was confirmed through an experiment involving a 25-day pot experiment conducted on green gram (*Vigna radiata*). While visible growth parameters such as plant height and leaf length did show an increase that failed statistical significance through ANOVA testing, there was a highly significant increase observed in the biomass dry weight ($P=0.00255$). This proves the capability of the biofertilizer to effectively promote internal nutrient assimilation and overall structure strength rather than external elongation. This is evidenced by the soil analysis conducted after harvest, which revealed higher levels of chemical soil fertility with the use of biofertilizer increasing available nitrogen by 9.51%, potassium by 12.52%, and phosphorus by 25% compared to control samples.

Conclusion

Demand of food is increasing day by day as population is increasing, so, proportionally food production should increase. But soil salinity, presence of heavy metals and inability of phosphate solubilization is a major problem.

Also, excessive uses of chemical fertilizers leads to soil fertility, harm normal soil microbes etc and can cause environment pollution. So, a Phosphate solubilizing bacterium like AM1 (*Acinetobacter sp. strain AM1*) that solubilize phosphorus in orthophosphate which plant can take up easily and have ability to produce organic acid, IAA and enzyme like phosphatase that has enormous role in plant growth promotion can act as biofertilizer. The present investigation is significant and relevant as the bacteria AM1 is able to solubilize insoluble phosphate to soluble one with simultaneous plant growth promoting hormone producing effects. This strain can increase in plant height and leaf length when sawdust was used as carrier. This increase in plant height, leaf length, soil N, P, K content indicates that this strain may be used as potential biofertilizer in not only in normal soil but also in saline soil therefore aiding sustainable agriculture in harsh environment. Also, this Phosphate solubilizing bacteria act as environmentally friendly, cost effective biofertilizer that is advantageous in many ways.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The data involving the organism are available in NCBI GenBank Database (OR430119, <https://www.ncbi.nlm.nih.gov/nuccore/OR430119.1/>). The data generated during &/or analyzed during the study are available from the corresponding author on reasonable request. Data will be made available on request.

Ethics statement

This research did not involve human participants, animal, subjects, or any materials that require the ethical approval.

Informed Consent Statement

This research did not involve human participants, animal, subjects, or any materials that require the ethical approval

Permission to Reproduce Material from other Sources

Not applicable.

Author Contributions

- **Ahana Mondal and Manasi Upadhyay:** Experiment, Validation, Data analysis and Resources.
- **Biswajit Saha:** Supervision, Conceptualization, Data analysis, Writing original draft, review and editing, resources.

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