Journal of Applied and Natural Science
17(1), 105 - 116 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Isolation and identification of phosphate-solubilizing *Bacillus sonorensis* GG2 from a mangrove ecosystem in Sundarban and its stimulating effect on green chili plant (*Capsicum frutescens* L.) growth

Palash Ghorai
Microbial Engineering and Algal Biotechnology Laboratory, Department of Biosciences, JIS University, Kolkata- 700109 (West Bengal), India
Dipankar Ghosh*
Microbial Engineering and Algal Biotechnology Laboratory, Department of Biosciences, JIS University, Kolkata- 700109 (West Bengal), India

*Corresponding author. E-mail: dghosh.jisuniversity@gmail.com

Article Info
https://doi.org/10.31018/jans.v17i1.5851
Received: June 10, 2024
Revised: January 29, 2025
Accepted: February 05, 2025

How to Cite
Ghorai, P. and Ghosh, D. (2025). Isolation and identification of phosphate-solubilizing *Bacillus sonorensis* GG2 from a mangrove ecosystem in Sundarban and its stimulating effect on green chili plant (*Capsicum frutescens* L.) growth. *Journal of Applied and Natural Science*, 17(1), 105 - 116. https://doi.org/10.31018/jans.v17i1.5851

Abstract
Phosphate-solubilizing bacteria (PSB) play a crucial role in enhancing soil fertility and plant growth by converting insoluble phosphates into forms accessible to plants. This study focused on isolating and identifying a phosphate-solubilizing bacterium, *Bacillus sonorensis* GG2, from the mangrove ecosystems of Sundarban. Through a series of microbiological and molecular techniques, *B. sonorensis* GG2 was identified and characterized for its phosphate solubilization potential. The isolate exhibited significant phosphate solubilization activity, which was quantified through in vitro assays. The bacterium was applied to green chili plants (*Capsicum frutescens* L.) under controlled conditions to evaluate its practical applicability. The results revealed a marked improvement in plant growth parameters, including root and shoot length, biomass, and overall plant health. In this study, two months of treatment of the green chilli plants with *B. sonorensis* GG2 positively affected the growth of the roots and shoot lengths compared with the control by 9.8% and 10.9%, respectively. This study highlights the potential of *B. sonorensis* GG2 as a biofertilizer, particularly in phosphate-deficient soils, and its role in sustainable agricultural practices.

Keywords: Orthophosphate, Phosphatase enzyme, Phosphate-solubilizing bacteria, Plant growth, Sustainable agriculture

INTRODUCTION

Phosphorus (P) is a significant ingredient for seed germination and plant growth. It is approximately 0.2% of a plant's dry weight (Alori *et al.,* 2017). Phosphorus is essential for various biological functions, including membrane structure maintenance, biomolecule synthesis, and the production of high-energy molecules. It also aids cell division, enzyme activation and inactivation, and glucose metabolism (Malhotra *et al.,* 2018). Phosphorus is a component of several energy-rich chemicals, including adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), phosphoenolpyruvate, and other phosphorylated intermediate compounds (Deng and Walther, 2020). Consequently, it provides energy to power numerous cellular endergonic activities. It is required for reproduction and protein synthesis since it is an element of nucleic acids (DNA and RNA) (Malhotra *et al.,* 2018).

Inorganic agrochemicals and soil microorganisms are two traditional modes of making phosphorus available to plants. The long-term use of large amounts of inorganic agrochemicals has a detrimental impact on soil fertility and plant production. It also contributes to environmental contamination and harms human health. Furthermore, deteriorating soil quality and the high expense of inorganic agrochemicals have increased daily agricultural production costs (Aryal *et al.,* 2021; Dogbatse *et al.,* 2021). In this current situation, phosphate-solubilizing bacteria are more sustainable for agricultural application as a fertilizer (Mussarat *et al.,* 2021). Soil holds maximum insoluble inorganic and organic phosphate in the precepted form. Plants only access soluble phosphate forms (i.e., orthophosphate) as a nutrient in the presence of water. Generally, only 0.1% of soil

phosphorus is accessible for plant use (Alori *et al.,* 2017; Ghosh *et al.,* 2022). However, P-solubilizing bacteria are widely used to promote plant growth by converting insoluble phosphate into orthophosphate (i.e., H_2PO_4 and HPO_4^{2-} ions), which is easily absorbed and utilized by roots (Krouk and Kiba, 2020)**.** Many bacteria, including *Bacillus megaterium*, *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Priestia megaterium, Rhizobium meliloti, Acinetobacter* sp., *Alcaligenes aquatilis, Azospirillum brasilense, Burkholderia cepacia, Paenibacillus macerans, Cytobacillus firmus,* and *Bacillus licheniformis,* are able to solubilize phosphate (Ghorai and Ghosh, 2022a; Suleman *et al.,* 2018). These precepted insoluble phosphates are recycled to soluble phosphate by phosphatase enzyme (i.e., acid phosphatase, alkaline phosphatase, phytase) synthesis and the generation of several forms of organic acids (i.e., citric acid, acetic acid, lactic acid, aspartic acid, maleic acid, succinic acid, gluconic acid) (Ghorai and Ghosh, 2022a; Tian *et al.,* 2021). The excretion of these organic acids causes a reduction in pH. As a result, the surrounding environment becomes acidic. As a consequence, P ions are liberated by exchanging H^+. It chelates the cations bound to phosphate, making it accessible to plants (Alori *et al.,* 2017; Li *et al.,* 2015).

The employment of biofertilizers in sustainable agricultural techniques necessitates a low concentration and high quality of bacterial inoculants in the soil, which substantially impact plant development. In the present situation, finding P-solubilizing bacteria that might be utilized as crop inoculants has limited success (Berninger *et al.,* 2018; Aloo *et al.,* 2022). According to this scenario, this study aims to identify and isolate the most potential P-solubilizing bacteria from a diverse ecosystem, such as the Sundarban mangrove ecosystem in West Bengal (Das and De, 2018). Sundarban mangrove soil contains stress elements such as SiO_4, K, and NaCl. The stress-tolerant bacterial community can flourish there due to the convergence of land and water (rivers and sea). This soil's present high phosphate concentration is directly governed by soil phosphatase activity (Biswas *et al.,* 2021). This study aimed to investigate the effectiveness of *B. sonorensis* GG2 as a P-solubilizing bacterium by evaluating its pathogenicity, stress resistance (halo-tolerance), and carrier viability. Additionally, this study compared culture-dependent and culture-independent methods to assess the growth of green chili plants (*Capsicum frutescens* L.) using this newly isolated phosphate-solubilizing bacterium *B. sonorensis* GG2.

MATERIALS AND METHODS

Sample collection

The Sundarbans mangrove forest is a diverse mangrove region in West Bengal. Three separate locations on the Sundarban River beach (a water-floating region): Dobanki (Bidyadhari River beach: 21.988228^0N, 88.753554^0E), Pakhiralay (Datta River beach: 22.140086^0N, 88.843076^0E) and Mathurakhanda (Junction of Bidyadhari River & Datta River beach: 22.058277^0N, 88.738887^0E) were used to collect soil samples (Fig. 1). The adhered soil samples were transported within 24 hours to the MEAB laboratory of JIS University, Kolkata, India, in ice conditions (4°C).

Screening and isolation of phosphate-solubilizing bacteria

Collected soil samples were mixed thoroughly in phosphate buffer with a pH of 7.2. After that, the P-solubilizing bacteria were isolated by the spread plate

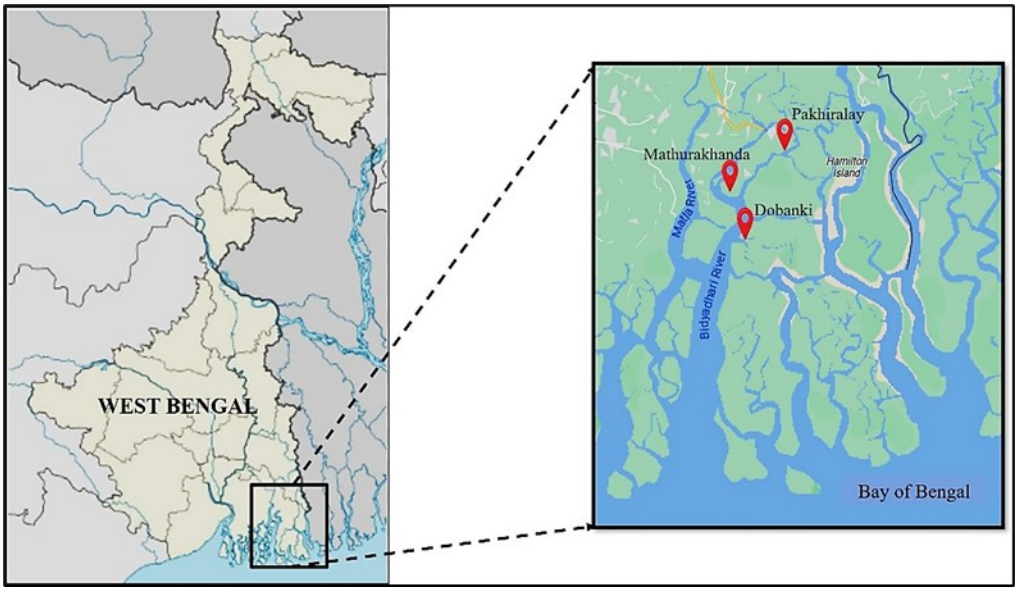

Fig. 1. *Map and geographical location of collected soil samples from Sundarban mangrove ecosystem.*

Table 1. Green chili plant growth under phosphate biofertilizer (*Bacillus sonorensis* GG2) treatment at 2 months (n=3).

Study sample character	Shoot heigh (cm)	Shoot diame-ter (cm)	Root length (cm)	No of branch root	No. of leaf	Leaf height (cm)	Leaf width (cm)
Control	10.4	0.4	12.6	16	7	4.9	1.8
P-biofertilizer	11.5	0.6	13.8	34	10	6.2	2.6

technique using selective Pvk (Pikovskaya) media (composition: glucose 10 gm, $CaHPO_4$ 5 gm, NaCl 0.2 gm, $MgSO_4.7H_2O$ 0.1 gm, KCl-0.2 gm, $(NH_4)_2SO_4$ 0.5 gm, $MnSO_4$. H_2O 0.002 gm, $FeSO_4.7H_2O$ 0.002 gm, yeast extract 0.5 gm, agar powder 16 gm in 1 liter of distilled water (D.W.)) (Ghorai and Ghosh, 2022b). After three days of incubation at 37°C, growing bacterial samples exhibited holo-zone formation. Six different P-solubilizing bacterial single-cell colonies were isolated and grown in different conical plates in Pvk broth media for future use. Six isolates were named, i.e., P1, P2, P3, P4, P5, and P6.

Six P-solubilizing bacterial isolates were analyzed in the exponential growth phase in Pvk selective media with inoculation in 1% (v/v) culture at 37°C in a BOD shaking incubator at 120 rpm. During a 50-hour growth investigation, a spectrophotometer (Shimadzu, Model: UV-1800) was used to assess optical density at 600 nm. After that, the exponential phase was identified to accelerate the qualitative potentiality check on Pvk agar plates using the Well diffusion agar plate method (Chen and Liu, 2019; Liu *et al.,* 2015). The action-equal biomass of the exponential growth phase culture of the phosphate-solubilizing bacterial sample was transferred aseptically to Pvk agar medium. After 72 h of incubation at 37°C, the holo-zone was observed in a Pvk medium plate.

Determination of the isolated phosphate-solubilizing bacterial cellular response

The phosphate-solubilizing bacterial Pvk broth culture (in the exponential growth phase) was centrifuged at 10,000 rpm for 10 minutes. Afterwards, the remaining supernatant was removed. The next intracellular reaction was studied using a crude extract of P-solubilizing bacterial cell pellet (sonicated in Phosphate-buffered saline (PBS)) transferred to Pvk agar plate medium. The extracellular response was studied by transferring a P-solubilized bacterial cell pellet (without sonication) to Pvk agar medium with PBS buffer. The Pvk agar plate exhibited a holo-zone after 72 hours of incubation at 37°C (Ghorai and Ghosh, 2023).

Estimation of orthophosphate productivity by the modified ascorbic method

Phosphate-solubilizing bacteria solubilize insoluble phosphate to generate orthophosphate. Five days of treatment of isolated PSB samples (inoculated 1% (w/v) exponential phase PSB biomass (mg) in Pvk broth (ml)) producing orthophosphate was estimated using a modified ascorbic acid technique by Ghorai and Ghosh (2022b).

Determination of phosphatase enzymatic activity using biochemical process

The pNP (para nitrophenol) measurement technique was used to calculate the phosphatase enzymatic activity. Phosphatase enzymes produce para-nitrophenol (pNP) by breaking para-nitrophenol phosphate (pNPP). After that, para-nitrophenol (pNP) reaction with alkaline solution (i.e., NaOH) forms a yellow color (i.e., p-nitrophenolate) (Kumar *et al.,* 2016).

For standard curve estimation, different para-nitrophenol concentrations were freshly prepared with the following working stock concentrations, i.e., 5 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm, by 4-nitrophenol (chemical name of pNP). A quantitative estimate was carried out by mixing 1 ml of 4-nitrophenol stock solution in 1 ml of 1 M NaOH and allowing the reaction to form yellow. Finally, absorbance was measured at 430 nm.

To carry out the estimation of bacterial culture solutions, first, 10 ml of Pvk broth media (without phosphate source) was inoculated with 1% (w/v) exponential phase PSB biomass (mg) in Pvk broth (ml). Then, 1 ml of 10 mM para-nitrophenol phosphate (pNPP) was added as a substrate. The experiment was then incubated at 37°C for 5 days in a BOD shaking incubator at 120 rpm. After that, the para-nitrophenol concentration (phosphatase activity) was estimated, and the culture mixture was centrifuged for 5 minutes at 10000 rpm. Then, 1 ml of supernatant was collected in an Eppendorf tube and mixed with 1 ml of 1 M NaOH solution. Finally, the mixed solution developed a yellow color. Then, the absorbance was measured at 430 nm by a spectrophotometer (Kumar *et al.,* 2016).

Determination of phosphatase enzymatic activity by the Well diffusion method

The phosphatase enzymatic activity cross-checks of the best isolate (P5) were made using the Well diffusion Pvk agar plate method. Organic phosphate was solubilized by phosphatase enzymes (Ghorai and Ghosh, 2022a). This study used Pvk agar media (composition as glucose 10 gm, lecithin 5 gm, NaCl 0.2 gm, $MgSO_4.7H_2O$ 0.1 gm, KCl-0.2 gm, $(NH_4)_2SO_4$ 0.5 gm, $MnSO_4$. H_2O 0.002 gm, $FeSO_4.7H_2O$ 0.002 gm, yeast extract 0.5 gm, agar powder 16 gm in 1 liter of

distilled water), insoluble phosphate should be used as an organic source as lecithin (Teng *et al.,* 2019). However, P-solubilizing bacterial samples (P5) were transferred aseptically into wells on Pvk agar medium (use lecithin). After three days of incubation at 37°C, growing bacterial samples exhibited holo-zone formation.

Molecular characterization of potential P-solubilizing bacterium P5 (*Bacillus sonorensis* GG2)

The P5 bacterial isolate (pure culture) was harvested via centrifugation (10,000 rpm for 5 min), and the genomic DNA of the P5 bacterial isolate was extracted using a bacterial DNA isolation kit. Following that, gDNA was purified, and RNaseA treatment was performed using a bacterial genomic DNA purification kit (Cat. No. MB505-50PR). A Denovix DS-11 spectrophotometer was used to evaluate the purity and concentrations. Polymerase chain reaction (PCR) was used to amplify 16S rRNA gene clusters, using the universal forward primer from ('10F') 5′- AGTTTGATCATGGCTCAGATTG-3′ and the universal reverse primer from ('800R') 5′- TACCAGGGTATCTAATCC -3′ (Hirai *et al*., 2016; Sandström *et al*., 2001). These primer sets were utilized to clone and amplify ~800 bp fragments containing virtually all 16S rRNA sequences. For 50 µl of PCR, one microliter (≈ 500 ng template) of whole DNA was used, along with 25 µl of EmeraldAmp GT PCR Master Mix (2X Premix) and 2.5 µl of primers (concentration of 0.2 µM) (using the Bacterial 16S rDNA PCR Kit Fast (800), Cat. No. # RR310A). To achieve a final volume of 50 µl, the necessary quantity of PCR dH_2O was added. The PCR conditions were as follows: 98°C for 10 s, 60°C for 30 s, and 72°C for 1 min for 30 reaction cycles in a thermal cycler (Lablife) (Franco-Duarte *et al.,* 2019; Weisburg *et al.,* 1991).

Following the completion of the PCR, the reaction mixture was electrophoresed on a 2% agarose gel (Fig. S1). The sample DNA solution and the positive control (*E. coli*) both produced amplification products of approximately 800 bp. Electrophoresis was used to determine the amplification products, which were then purified for sequencing analysis. A GeneJET Extraction Kit (Cat. No. #K0691) was used to purify the leftover reaction mixture after PCR produced a single ~800 bp band. After purification, the amount of purified PCR products was evaluated by measuring their absorbance ratio (A_{260}:A_{280}). Then, sequencing was performed with an ABI 3730 Genetic Analyzer using the original PCR primers (Ghorai and Ghosh, 2023).

***Bacillus sonorensis* GG2 pathogenicity test by hemolysis assay**

A pathogenicity test for *B. sonorensis* GG2 was performed via a hemolysis assay. *B. sonorensis* GG2 (exponential phase culture) was inoculated in blood agar-based selective media (containing 5% v/v defibrinated sheep blood) under a sterile microenvironment to conduct the pathogenicity evaluation step following Ghorai and Ghosh (2023).

***Bacillus sonorensis* GG2 bacterial salinity tolerance efficiency study**

Bacillus sonorensis GG2 was inoculated in 1% (v/v) culture on YP culture medium (2 g/l yeast extract, 5 g/l peptone) with different concentrations (i.e., 0, 25, 50, 75, and 100 g/l) of NaCl. Cells were cultivated for 5 days at 37°C and 120 rpm orbital agitation. OD_{600} was measured using a UV–visible spectrophotometer to track growth (Remonsellez *et al.,* 2018).

***Bacillus sonorensis* GG2 viability study in carriers**

Lignite and cow dunk compost (CDC) were obtained as carrier materials from the MSV Laboratories Pvt. Ltd. in West Bengal (a biofertilizer company). This can occur while maintaining the physicochemical parameters of the two carriers, such as pH and moisture. Carrier material pH 7 was maintained by adding $CaCO_3$. Similarly, drying with hot air keeps the moisture at approximately 20-30% (Bello *et al.,* 2019; Gade *et al*., 2014). The carrier material was then sterilized by autoclaving at 121°C for 1 hour at 15 pounds per square inch pressure. After that, four inoculated bags were prepared as follows: (i) CDC-control (without mixing any isolates in the CDC); (ii) CDC-GG2 (*B. sonorensis* GG2 had been inoculated in the CDC carrier); (iii) lignite-control (without mixing any isolates in lignite); and (iv) lignite-GG2 (inoculated with *B. sonorensis* GG2 in lignite in the carrier). The inoculation concentration of *B. sonorensis* GG2 was 5 ml of exponential phase culture (1x 10^{12} cfu/ml) per kg of the carrier on laminar airflow (Abd El-Fattah *et al.,* 2013). After inoculation, *B. sonorensis* GG2 in carriers was stored at room temperature (~ 25–30°C) for 180 days.

The spread plate approach was used to test the ability of live phosphate-solubilizing *B. sonorensis* GG2 bacteria in the carrier (CDC & lignite) at 30-day intervals for up to six months on days 0, 30, 60, 90, 120, 150, and 180. For the spread plate viability test, 1 g of the treated carrier was combined with 10 mL of sterile distilled water and serially diluted to 10^{-6} in a laminar manner. Then, 50 µL of diluted culture was placed on a nutrient agar plate. After one day of incubation at 37°C in a BOD incubator, the bacterial colonies expanded. The colonies were then enumerated manually, and the findings were represented as colony-forming units per gram (cfu/gm), using Equation (1) (Gade *et al.,* 2014).

$$\text{c. f. u./gm} = \frac{(\text{No.of colony} - \text{No.of control colony}) \times \text{dilution}}{\text{Volume(in ml)}} \quad \text{... (Eq. 1)}$$

Plant growth study

For the study on plant growth, soil samples were taken

from the Pataspur area (21.984060°N, 87.462378°E). It is one of the most significant agricultural areas in West Bengal's Purba Medinipur district. Clayey soils dominate nature in this area (Sahu, 2014).

In this study, *B. sonorensis* GG2 phosphate-solubilizing bacteria were used for rooftop gardening to harvest green chili plants (*Capsicum frutescens* L.), and their above- and belowground growth characteristics were examined during the spring season (temperature 28 (± 2) °C) (Raza *et al.,* 2021; Bhutia *et al.,* 2018). Two times, the *B. sonorensis* GG2 strain was used as a soil inoculum. First, 2 ml of *B. sonorensis* GG2 was mixed with 10 g of green chili seed 30 minutes before seed germination. After ten days of seeding, 1 gm of dry biomass was inoculated into soil with a mix of water. After two months of plant growth, parameters such as plant shoot and root morphology, number of secondary roots, number of leaves and leaf morphology were measured.

Statistical analysis

Statistical analysis was executed using IBM SPSS software (version 26.0). Two correlation coefficient tests (Pearson Correlation) were performed to confirm the significant correlation at 0.05 significance levels (2-tailed) among the (a) day, biomass concentration, orthophosphate concentration, and (b) day, biomass concentration, para-nitrophenol concentration (phosphatase activity) of *B. sonorensis* GG2 in Pvk sterile medium for small-scale experiments (Debnath and Ghosh, 2023).

RESULTS AND DISCUSSION

In the present study, after 72 hours of incubation, white –grey single-cell colony formation on Pvk agar medium and slight holo-zone formation were observed. The study obtained essential entities and six effective P-solubilizing bacterial single-cell colony isolates (nomenclatures such as P1, P2, P3, P4, P5, and P6) (Fig. 2a and b). This study is one of the few explorations of PSB from mangrove ecosystem. Mangroves are dynamic and stress-prone environments, offering a unique niche for microbial diversity.

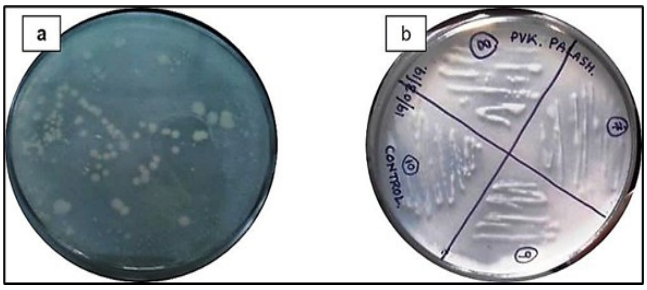

Fig. 2. *Screening and isolation of P-solubilizing bacteria (a) Soil sample spread in Pvk media (one best plate shows), (b) Restreak of a single cell colony in Pvk media (one best plate shows).*

Optimization of the qualitative potentiality study by the Well diffusion method

Six P-solubilizing bacterial exponential phases were examined in Pvk selective medium as inoculums for comparative investigation. The exponential phase was observed in Pvk selective medium at 25 hours for the P1 isolate, 30 hours for the P2 isolate, 30 hours for the P3 isolate, 35 hours for the P4 isolate, 35 hours for the P5 isolate, and 30 hours for the P6 isolate (Fig. S2). Then, inoculating the same number of P-solubilizing bacteria in exponential conditions showed excellent P-solubilizing ability in Pvk selective agar medium by clear holo-zone formation. The three isolates with the greatest outcomes in this investigation were P1, P3, and P5 (Fig. 3 & Fig. S3).

Determination of the isolated phosphate-solubilizing bacterial cellular response

All bacterial isolates showed extracellular activity for phosphate solubilization by holo-zone creation in Pvk agar media (Fig. S4). The extracellular response was quite effective for soil application because the extracellular response exposed the maximum reaction shown outside the cell.

Comparative study of orthophosphate production

Compared to the control (colorless), the modified ascorbic approach used to isolate samples shows a significant amount of orthophosphate generation capacity in Pvk liquid media by blue color development (Fig. 4b and 3c). Experiment P5 isolates performed better in phosphate solubilization than the other five isolates (Fig. 4a). However, the P5 isolate specifically showed higher orthophosphate productivity at 0.0021 mg. ml^{-1}. day^{-1} with 2.114 mg. ml^{-1}. day^{-1} biomass developed. During the five-day experiment, a pH drop (i.e., pH 7.2 to ≈pH 4) was recorded in six P-solubilizing culture inoculates (Fig. 4d). This primarily ensures that P-solubilizing bacterial isolates synthesize organic acids.

Determination of phosphatase activity

The standard curve in the para-nitrophenol method produced a very accurate result when the R^2 value was 0.997 (Fig. 5a). Inoculation of the P-solubilizing bacteria resulted in significant phosphatase activity by para-nitrophenol phosphate (pNPP) to para-nitrophenol (pNP) production with yellow color formation. This observation demonstrates that the best results shown in this experiment are P2 and P5 isolates (Fig. 5b-d). However, the P5 isolate specifically showed that para-nitrophenol productivity (i.e., phosphatase activity) was 0.00192 mg. ml^{-1}. day^{-1} with 1.667 mg. ml^{-1}. day^{-1} bio-

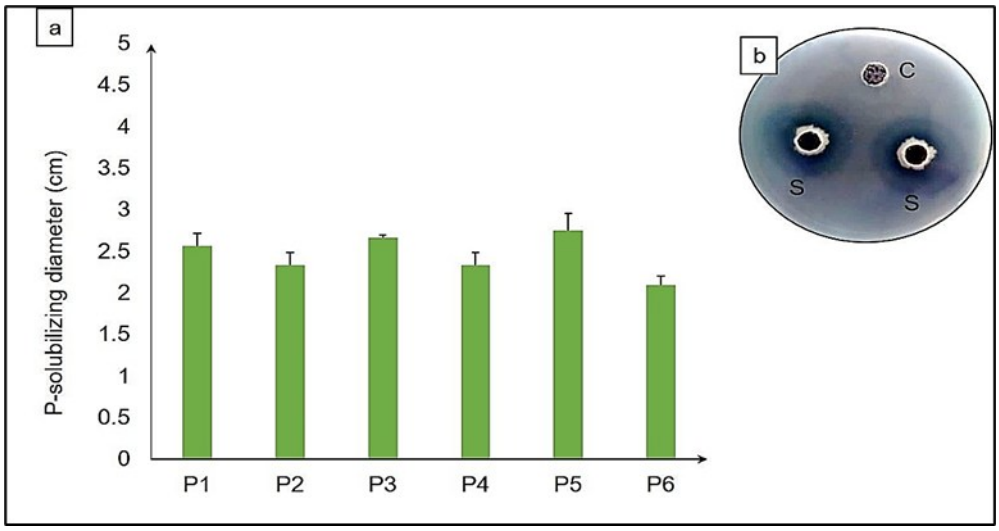

Fig. 3. *Qualitative study of P-solubilizing isolates (a) Comparison of P-solubilizing ability by holo-zone diameter (n=3), (b) one best plate shows in a qualitative study (C: control; S: pure culture).*

mass developed. Additionally, the P2 isolate specifically showed that para-nitrophenol productivity (phosphatase activity) was 0.00193 $mg.ml^{-1}.day^{-1}$ with 1.801 $mg.ml^{-1}.day^{-1}$ biomass developed. These studies showed that P2 isolates use higher biomass to give the same phosphatase activity. Phosphatase activity profile studies showed that the P5 isolate gives comparatively higher productivity than the P2 isolate. Additionally, the best isolate P5 results show insoluble lecithin (organic phosphate) degradation activity by holo-zone creation in the Pvk agar plate. It ensured phosphatase activity on the P5 bacterial isolate (Fig. 5e).

Despite the *Bacillus sonorensis* GG2 strain's impressive phosphatase and orthophosphate-producing activities, its overall phosphate-solubilizing activity was found to be significantly low. This limitation affects its potential effectiveness as a biofertilizer compared to other phosphate-solubilizing bacterial (PSB) isolates.

This study confirmed the phosphate solubilization ability of the bacterial isolate P5 through holo-based techniques and biochemical estimations. Additionally, the P5 strain exhibited low capability in its potential effectiveness as a biofertilizer compared with other phosphate-solubilizing bacterial isolates (Table ST1). This comparison should highlight the unique contributions and advantages of the current study.

Bacillus sonorensis GG2 bacterial phylogenetic analysis

The genetic analyzer identified the consensus sequence of the 16S rRNA-encoding gene cluster of the P5 isolate generated by BLAST analysis to compare with the sequences in the NCBI GenBank database. The P5 isolate was highly similar (96.74%) to *B. sonorensis* (Fig. S5). The new isolate was named and abbreviated as *B. sonorensis* GG2. Furthermore, the 16S

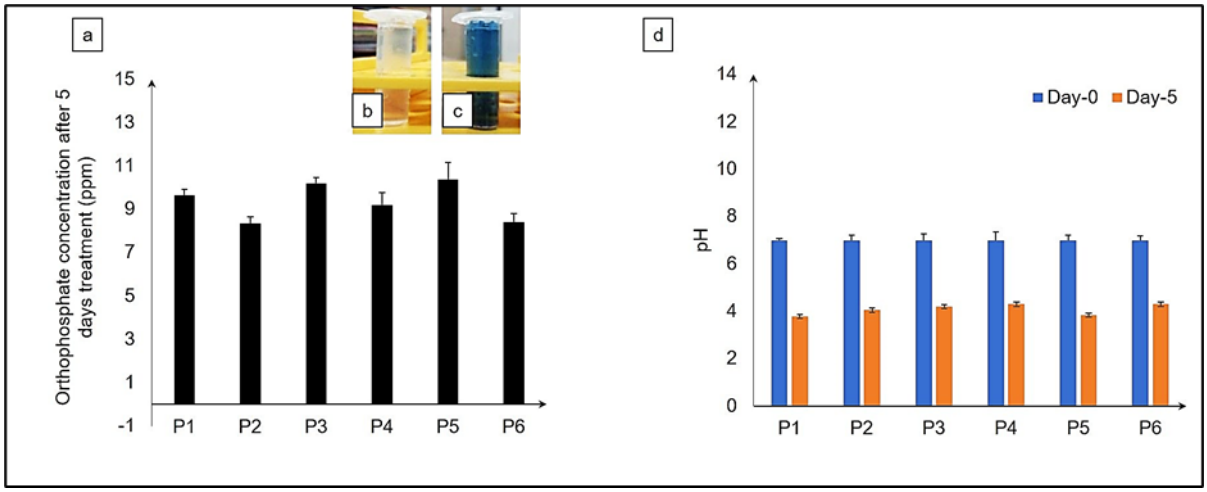

Fig. 4. *Quantitative study of P-solubilizing isolates (a) Comparative study of orthophosphate production after 5 days of treatment in Pvk broth, (b) Control sample color of the orthophosphate quantification test using the modified ascorbic acid method (no color change), (c) Blue color shows the best orthophosphate-producing isolate (P5 isolate) using the ascorbic acid method, and (d) Measurement of pH change by Pvk media treatment at 5 days.*

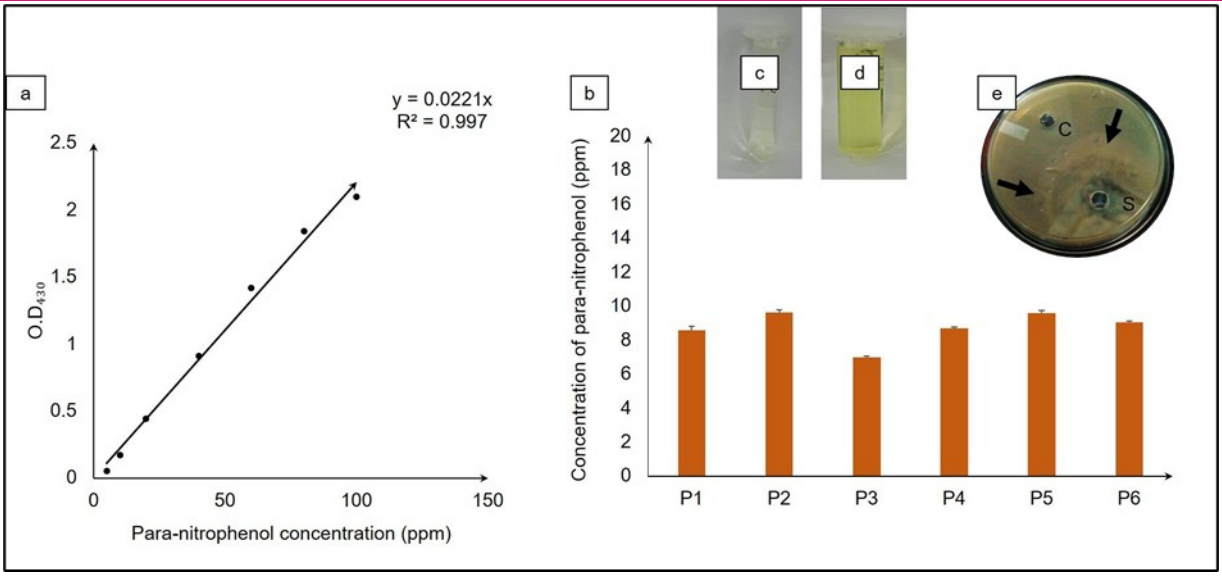

Fig. 5. *Quantitative analysis of phosphatase enzymatic activity (a) standard curve of para-nitrophenol, (b) comparative analysis of para-nitrophenol for determining phosphatase enzymatic activity after 5 days of treatment in Pvk media, (c) control of the phosphatase activity test (no color change), (d) yellow color shows the best isolate (P5 isolate) of the phosphatase activity test, and (e) organic phosphate solubilizing ability check by the well diffusion method (C: control; S: pure culture).*

rRNA nucleotide sequences from *B. sonorensis* GG2 were compared with other popular P-solubilizing bacterial sequences in the databases using multiple sequence alignment (Datafile SD1 & SD2) to infer an integrated phylogenetic relationship. A phylogenetic tree representing fourteen major groups of P-solubilizing 16S rRNA sequences was generated and is shown in Fig. 6. *Bacillus sonorensis* GG2 has a very close molecular character to the other prudential P-solubilizing bacterial strains, likely *Bacillus licheniformis* SQR2 and *Cytobacillus firmus* NPBR2.

Bacillus sonorensis GG2 pathogenicity test

Hemolysis assays are commonly used to assess the ability of bacteria to lyse red blood cells, which is an indicator of pathogenic potential. Compared to the positive control, none of the selected *B. sonorensis* GG2 strains developed blood lysis or holo-zones on blood agar medium (Fig. 7a). On the other hand, in the broth, *B. sonorensis* GG2 hemolysin activity was very low (less than 10%) (Fig. 7b-e). The hemolysis assay confirmed that *B. sonorensis* GG2 is non-toxic to humans, ensuring its safety for use in agricultural practices.

Salinity tolerance of *Bacillus sonorensis* GG2 bacteria

The growth curve of the *B. sonorensis* GG2 strain demonstrates its capacity to reproduce in the face of significant salt concentrations. This strain displays its greatest growth behavior after five days of treatment at 25 g/l NaCl. It also grew slightly slowly in the 50 and 75 g/l NaCl concentrations. Finally, *B. sonorensis* GG2 very low survive and reproduce at 100 g/l in extremely high NaCl conditions (Fig. 8). *B. sonorensis* GG2 exhibited high salt tolerance, up to 75 g/l NaCl, which is notably higher than many other PSB strains (Table ST1). This makes it particularly useful for application in saline soils, where other strains might fail.

Viability study in the CDC and lignite carrier

Commercializing the best *B. sonorensis* GG2 bacteria isolate for researching six-month viability in a specified carrier such as lignite and CDC. This study showed that after 180 days of storage treatment, carrier CDC and lignite held at room temperature had the maximum viable cell number of P-solubilizing bacteria (Fig. 9a; Fig. S6). The *B. sonorensis* GG2 strain had the maximum number of live bacterial cells in the lignite carrier on day 30. The study demonstrated that *B. sonorensis* GG2 maintained high viability in CDC (16.5%) and lignite (32.7%) carriers for up to six months. This long-term viability in carriers is crucial for the practical application of PSB as bioinoculants, ensuring their effectiveness over extended periods (Fig. 9b).

Effects of *Bacillus sonorensis* GG2 on green chili plant shoot height and diameter

In the present study, phosphate affected the morphology of the chili plant, including shoot development (Fig. 10a and b). The shoot height of the green chili plant treated with *B. sonorensis* GG2 was measured 60 days following seedling emergence and is depicted in Table 1. After being treated with *B. sonorensis* GG2, the chili plant produced shoots that grew 11.5 cm tall and 0.6 cm in diameter. The control group (no biofertilizer usage) had the lowest shoot height and diameter meas-

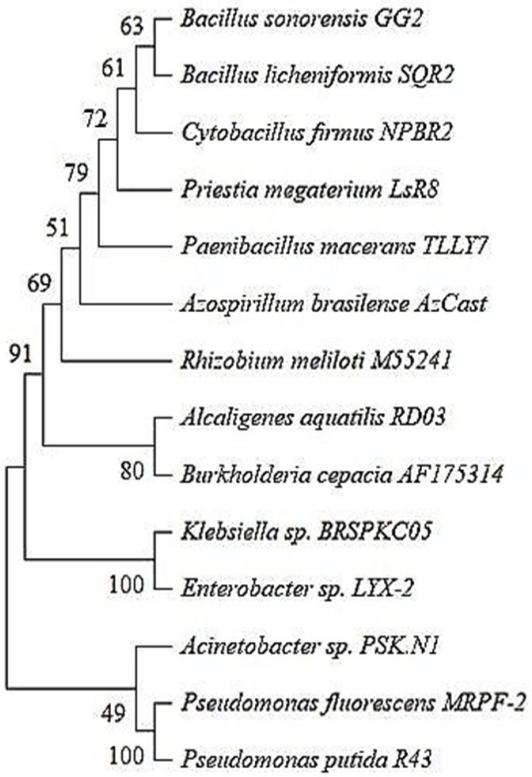

Fig. 6. *Multiple sequence alignments for molecular phylogenetic analysis were generated using SnapGene and optimized using MEGA11. Nearest-Neighbor-Interchange (NNI) method, Tamura-Nei model and Maximum Likelihood (ML) statistical models were used to infer the phylogenetic relationship among strains of closely associated P-solubilizing bacteria based on 16S rRNA gene sequences. A substitution model for phylogenetics of each nucleotide sequence data set was selected using the Tamura-Nei model with model selection in MEGA11. Representative phylogenetic trees were drawn using the NNI and ML methods (Saitou and Nei, 1987; Felsenstein, 1995).*

urements, measuring 10.4 cm and 0.4 cm, respectively. On the basis of actual P solubilization findings from *B. sonorensis* GG2 on chili plants, a significant increase in shoot diameter (58.3% increase) and height (10.9% increase) was observed compared with those of the control.

Effect of *Bacillus sonorensis* GG2 on chili root length and number of branches

Bacillus sonorensis GG2 treatments with phosphate-solubilizing biofertilizer substantially impacted the shape of green chili roots (Fig. 10b). In the *B. sonorensis* GG2 treatment, the length of the chili root was significantly increased by 13.8 cm from the nonfertilized condition of 12.6 cm. The *B. sonorensis* GG2-treated plant had 34 root branches (secondary root), whereas the untreated plant had 16 root branches. It is abundantly obvious from these data that *B. sonorensis* GG2 significantly affected the shape of plant roots. The effective results of *B. sonorensis* GG2 as a phosphate biofertilizer on chili plants showed considerable increases in root length (9.8% increase) and the number of secondary roots (114.58% increase) compared to control growth, as shown in Table 1.

Effects of *Bacillus sonorensis* GG2 on leaf number and morphology

Phosphate-solubilizing *B. sonorensis* GG2 treatments significantly influenced the green chili plant leaf number (Fig. 10a). Treatment with *B. sonorensis* GG2 resulted in more than three leaves compared with the treatment without fertilization (Table 1). This study's findings demonstrated that using *B. sonorensis* GG2 as a phosphate biofertilizer substantially impacted green chili plant development.

Treatments with *B. sonorensis* GG2 substantially impacted the leaf width and length (Fig. 10c). *Bacillus sonorensis* GG2 treatment resulted in chili plant leaves that were 6.2 cm in length and 2.6 cm in width. The lowest chili plant leaf growth was recorded in the control (without biofertilizer) at 4.9 cm (length) and 1.8 cm (width), as shown in Table 1. The treatment *B. sonorensis* GG2 as a P-biofertilization showed significant growth regarding leaf length (25.85% rise) and leaf width (49.05% rise) compared to the untreated plant. Furthermore, the effect of phosphate biofertilizer on *B. sonorensis* GG2 leaves was not substantially influenced by the color of the leaves. There were no greater differences across the treatments.

The results demonstrate the potential of *B. sonorensis* GG2 as a biofertilizer, particularly in phosphate-deficient soils. This aligns with the global push towards sustainable agricultural practices and reducing dependence on chemical fertilizers. The significant improvement in green chili plant growth observed in this research suggests that *B. sonorensis* GG2 might possess significance phosphate solubilization abilities or additional plant growth-promoting traits, warranting further investigation.

Statistical analysis of *Bacillus sonorensis* GG2 P-solubilizing profile with biomass growth

P-solubilizing profile studies have shown that *B. sonorensis* GG2 gives comparatively higher productivity (orthophosphate 0.0021 mg. ml^{-1}. day^{-1} & para-nitrophenol 0.0019 mg. ml^{-1}. day^{-1}) compared to the other five isolates on five days of cultivation (Fig. 4a and 5b). Biomass, orthophosphate and pNP production profiles using *B. sonorensis* GG2 from mangrove ecosystems increased daily. Moreover, the experimental results showed that orthophosphate and pNP production is proportional to the biomass production of the *B. sonorensis* GG2 strain (Fig. S7). However, statistical analysis of the correlation coefficient test was per-

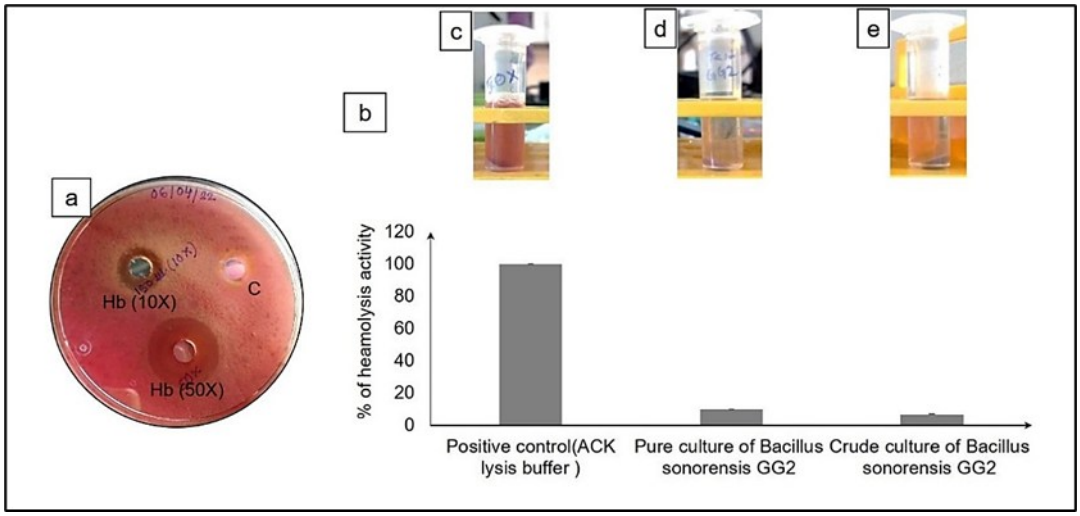

Fig. 7. *Bacillus sonorensis GG2 bacterial hemolysis assay (a) hemolysis assay in sheep blood agar plates with Hb: ACK lysis buffer as a positive control (different concentrations of 10X & 50X), C: Bacillus sonorensis GG2 pure culture, (b) comparative study of hemolysin activity in sheep blood broth media (n=3), (c) hemolysin activity of ACK lysis buffer, (d) hemolysin activity of B. sonorensis GG2 pure culture, (e) hemolysin activity of B. sonorensis GG2 crude culture.*

formed to confirm a significant correlation among the (a) day, biomass concentration and orthophosphate concentration (Table ST2) and (b) day, biomass concentration and concentration of pNP (phosphatase activity) (Table ST3) using IBM SPSS 26 statistical software at the 0.05 significance level. The results showed significant relations among all three parameters in *B. sonorensis* GG2 strain assemblages.

Based on the present study, the eminent researchers have done different research that shows significant results. A detailed discussion has been done in the following section and in Table ST3. *Kulkova* et al. (2024) conducted a study where *Serratia* sp. strains exhibit plant growth-promoting traits, enhancing plant growth and tolerance to abiotic stresses like salinity and drought. These strains can alleviate salinity stress through 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase production, EPS (exopolysaccharide) production, osmolyte accumulation, and modulation of stress-related gene expression. Additionally, *Serratia* sp. can mitigate drought stress by producing ACC deaminase and EPS and inducing osmolyte and antioxidant accumulation. However, the study calls for further elucidation of the molecular mechanisms by which *Serratia* sp. promotes plant growth and the need to evaluate its effects on indigenous soil and plant microbiota, particularly in the rhizosphere. Before field trials, researchers must rule out the possibility of some *Serratia* sp. strains being phytopathogenic. More studies are needed to assess the impact of *Serratia* spp. on native rhizosphere microbiota across diverse plants, soils, and climate conditions.

Alvarez-Sastre et al. (2022) investigated desert-derived actinobacterial strains, which can tolerate extremely high salinity levels of up to 18% NaCl. These desert actinobacterial strains can also improve plant tolerance to salinity stress, similar to other types of bacteria like *Halomonas* and *Bacillus*. The study found that several desert-derived actinobacterial strains can tolerate salinity and improve plant tolerance to salinity stress. *Micromonospora* isolates did not have the highest salt tolerance compared to other genera like *Micrococcus*, even though they were still able to improve plant growth and resilience to salt stress. The diversity of microorganisms in desert environments is still not well understood, suggesting a need for further research in this area. This study is a first step in exploring the use of desert-adapted microorganisms to improve plant resilience to climate change. However, more research is needed to realize this potential fully. According to

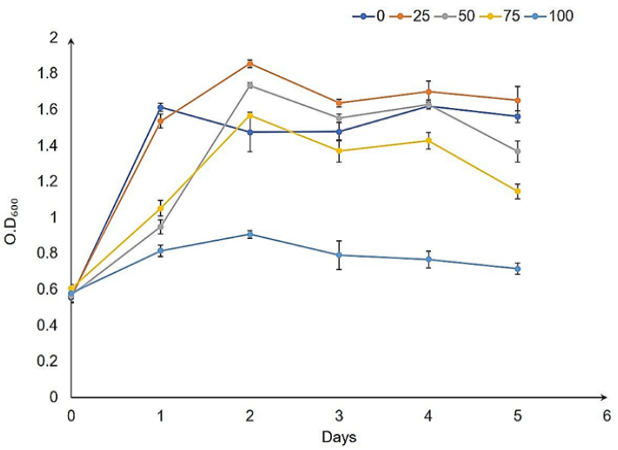

Fig. 8. *Growth of Bacillus sonorensis GG2 in the presence of NaCl. Bacillus sonorensis GG2 cells were grown in their respective growth media (YP media) in the absence of NaCl or supplemented with 25, 50, 75, and 100 g/l NaCl. OD_{600} readings were recorded for 5 days at 24-hour intervals (n = 3).*

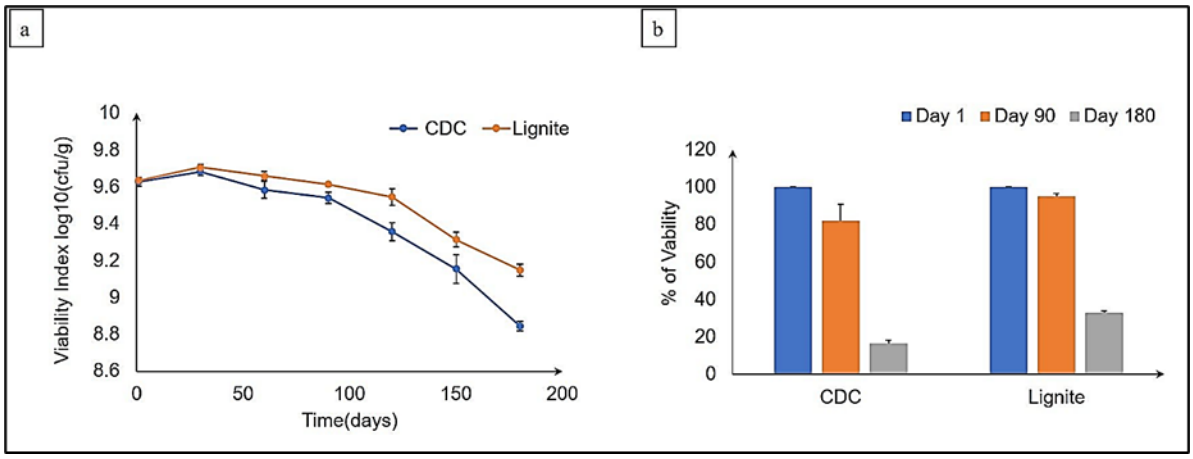

Fig. 9. *Viability study of Bacillus sonorensis GG2 in carrier (n=3) (a) 180 days viability study in lignite and CDC carrier using best P-solubilizing bacterial isolate B. sonorensis GG2, (b) comparative study percentage (%) of viability after 180 days treatment of B. sonorensis GG2 bacteria in CDC and lignite carriers.*

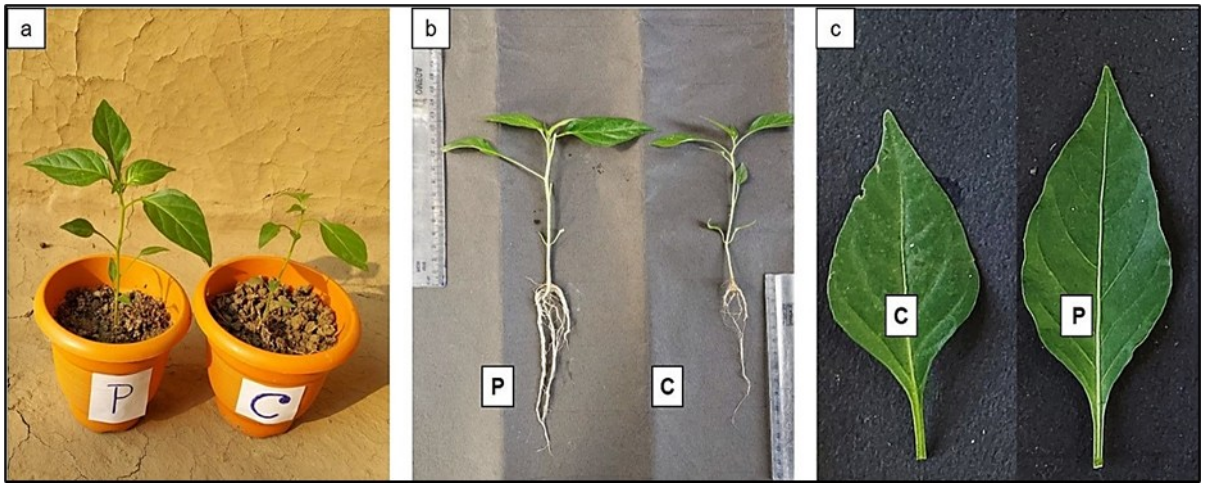

Fig. 10. *Showing a single green chili plant growth of phosphate biofertilizer (B. sonorensis GG2) treatment at 2 months (a) P: Bacillus sonorensis GG2 treated plant growth after 2 months, C: Untreated plant growth after 2 months, (b) P: Bacillus sonorensis GG2 treated plant root and shoot growth after 2 months, C: Untreated plant root and shoot growth after 2 months, (c) P: Bacillus sonorensis GG2 treated plant leaf growth (consider best leaf) after 2 months, C: Untreated leaf growth (consider best leaf) after 2 months.*

Nozari et al. (2021), all *Streptomyces* sp. produced indolic compounds (IC) and siderophores. Some *Streptomyces* isolates (6 out of 10) could produce ACC deaminase, while only two isolates produced phenazines. Two *Streptomyces* isolates, CLV97 and CLV179, significantly promoted maize plants' growth and alleviated salinity's negative effects on plant growth. The study only examined root colonization by *Streptomyces* sp. over a 20-day period and did not look at longer-term effects. Furthermore, the study focused on the effects of only two *Streptomyces* isolates (CLV97 and CLV179) and did not examine the effects of the other isolates in detail. It also did not fully characterize the production of other plant growth-promoting compounds beyond indolic compounds and siderophores.

In contrast to the above research studies, the present study found that the microbial system isolated from the Sundarban mangrove eco-system, that is, P5 isolate, *Bacillus sonorensis* GG2 produced 10 mg L^{-1} of a specific compound after five days, equating to a production rate of 0.0021 mg mL^{-1} day^{-1} and a biomass production rate of 2.11 mg mL^{-1} day^{-1} . This clearly indicates the stress tolerance capability of the isolated strain with a salinity tolerance level of 7.5-10%, along with acceptable phosphate solubilizing activity. The strain also shows phosphatase enzyme activity and organic acid productivity with a highly significant industrial application.

Conclusion

The isolation and characterization of *Bacillus sonorensis* GG2 from the Sundarban mangrove ecosystem and its demonstrated efficacy in promoting green chili plant growth contribute novel insights into the field of agricultural microbiology. By exploring a unique and underexplored environment, this study not only expands the known diversity of PSB but also highlights the practical

benefits of using such strains in sustainable agriculture. These promising results suggest that *B. sonorensis* GG2 could be a valuable addition to the arsenal of biofertilizers, offering a sustainable solution for enhancing crop productivity in phosphate-deficient soils. Therefore, in future studies, multiprocess parameter optimization of various independent variables and genetic modification must be carried out to increase the maximum phosphate solubilization efficacy and field potentiality. This can encourage environmentally friendly farming to help meet sustainable agricultural demands.

Supplementary Information

The author(s) is responsible for the content or functionality of any supplementary information. Any queries regarding this should be directed to the corresponding author. The supplementary information (S1-S7; ST1-3; SD1-2) is downloadable from the article's webpage and will not be printed in the print copy.

Conflict of interest

The authors declare that they do not have any conflicts of interest.

REFERENCES

1. Abd El-Fattah, D. A., Eweda, W. E., Zayed, M. S. & Hassanein, M. K. (2013). Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. *Annals of Agricultural Science*, 58(2), 111–118. https://doi.org/10.1016/j.aoas.2013.07.001
2. Aloo, B. N., Mbega, E. R., Makumba, B. A. & Tumuhairwe, J. B. (2022). Effects of carrier materials and storage temperatures on the viability and stability of three biofertilizer inoculants obtained from potato (*Solanum tuberosum* L.) rhizosphere. *Agriculture*, 12(2), 140. https://doi.org/10.3390/agriculture12020140
3. Alori, E. T., Glick, B. R. & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, 8, 971. https://doi.org/10.3389/fmicb.2017.00971
4. Alvarez-Sastre, C., & Carro, L. (2022). Desert actinobacterial strains increase salt stress resilience in crops. *Environmental Sciences Proceedings*, *16*(1), 17. https://doi.org/10.3390/environsciproc2022016017
5. Aryal, J. P., Sapkota, T. B., Krupnik, T. J., Rahut, D. B., Jat, M. L. & Stirling, C. M. (2021). Factors affecting farmers' use of organic and inorganic fertilizers in South Asia. *Environmental Science and Pollution Research International*, 28(37), 51480–51496. https://doi.org/10.1007/s11356-021-13975-7
6. Bello, A., Begianpuye, A., Alfa, A., Abdulraheem, G., Adamu, Y. & Anthony, D. (2019). Development of biofertilizer from composted cow-dung. *Science Forum: Journal of Pure and Applied Sciences*, 0, 1. https://doi.org/10.5455/sf.55585
7. Berninger, T., González López, Ó., Bejarano, A., Preininger, C. & Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. *Microbial Biotechnology*, 11(2), 277–301. https://doi.org/10.1111/1751-7915.12880
8. Bhutia L, K., Vk, K., Meetei NG, T. & Bhutia D, N. (2018). Effects of climate change on growth and development of Chilli. *Agrotechnology*, 07(02). https://doi.org/10.4172/2168-9881.1000180
9. Biswas, S.K., Zaman, S. & Das, M. (2021). Soil of Sundarban delta is rich in sodium, potassium, silicate and phosphorus. *Pharma Innovation*, 10(9),1754-1755.
10. Chen, Q. & Liu, S. (2019). Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in reclamation soil in Shanxi, China. *Frontiers in Microbiology*, 10, 2171. https://doi.org/10.3389/fmicb.2019.02171
11. Das, S. & De, T. K. (2018). Microbial assay of N2 fixation rate, a simple alternate for acetylene reduction assay. *MethodsX*, 5, 909–914. https://doi.org/10.1016/j.mex.2017.11.010
12. Debnath, S. & Ghosh, D. (2023). Mangrove algae as sustainable microbial cell factory for cellulosic biomass degradation and lipid production. *Journal of Applied Biology & Biotechnology*. 11(3), 160-171. https://doi.org/10.7324/jabb.2023.76846
13. Deng, J. & Walther, A. (2020). ATP-responsive and ATP-fueled self-assembling systems and materials. *Advanced Materials (Deerfield Beach, Fla.)*, 32(42), e2002629. https://doi.org/10.1002/adma.202002629
14. Dogbatse, J. A., Arthur, A., Awudzi, G. K., Quaye, A. K., Konlan, S. & Amaning, A. A. (2021). Effects of organic and inorganic fertilizers on growth and nutrient uptake by young cacao (*Theobroma cacao* L.). *International Journal of Agronomy*, 2021, 1–10. https://doi.org/10.1155/2021/5516928
15. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution; International Journal of Organic Evolution*, 39(4), 783. https://doi.org/10.2307/2408678
16. Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K. S., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Relison Tintino, S., Alexandrino de Souza, V. C., Sharifi-Rad, J., Coutinho, H. D. M., Martins, N. & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms-from past to present. *Microorganisms*, 7(5), 130. https://doi.org/10.3390/microorganisms7050130
17. Gade, R.M., Chaithanya, B.H. & Khurade. K.C. (2014). A comparitive study of different carriers for shelflife of *Pseudomonas fluorescens. Bioscan*, 9(1), 287-290.
18. Ghorai P, Ghosh D. (2023) Screening and isolation of potential nitrogen-fixing *Enterobacter* sp. GG1 from mangrove soil with its accelerated impact on green chili plant (*Capsicum frutescens* L.) growth amelioration. *Journal of Applied Biology & Biotechnology*, 1-9. https://doi.org/10.7324/JABB.2023.139674
19. Ghorai, P. & Ghosh, D. (2022a). Ameliorating the performance of NPK biofertilizers to attain sustainable agriculture with special emphasis on bioengineering. *Bioresource Technology Reports*, 19(101117), 101117. https://doi.org/10.1016/j.biteb.2022.101117

20. Ghorai, P. & Ghosh, D. (2022b). Sustainable approach for insoluble phosphate recycling from wastewater effluents. In: *Environmental Degradation: Monitoring, Assessment and Treatment Technologies* (pp. 77–86). Springer International Publishing. https://doi.org/10.1007/978-3-030-94148-2_7
21. Ghosh, D., Ghorai, P., Debnath, S., Indrama, T., Kondi, V. & Tiwari, O. N. (2022). Algal biofertilizer towards green sustainable agriculture. In: *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 27–45). Elsevier. https://doi.org/10.1016/B978-0-323-85579-2.00019-8
22. Hirai, J., Sakanashi, D., Hagihara, M., Haranaga, S., Uechi, K., Kato, H., Hamada, H., Nishiyama, N., Koizumi, Y., Suematsu, H., Yamagishi, Y., Fujita, J. & Mikamo, H. (2016). Bacteremia due to Streptococcus tigurinus: A case report and literature review. *Journal of Infection and Chemotherapy: Official Journal of the Japan Society of Chemotherapy*, 22(11), 762–766. https://doi.org/10.1016/j.jiac.2016.04.011
23. Krouk, G. & Kiba, T. (2020). Nitrogen and Phosphorus interactions in plants: from agronomic to physiological and molecular insights. *Current Opinion in Plant Biology*, 57, 104–109. https://doi.org/10.1016/j.pbi.2020.07.002
24. Kulkova, I., Wróbel, B., & Dobrzyński, J. (2024). *Serratia* spp. as plant growth-promoting bacteria alleviating salinity, drought, and nutrient imbalance stresses. *Frontiers in Microbiology*, *15*, 1342331. https://doi.org/10.3389/fmicb.2024.1342331
25. Kumar, S., Chauhan, P. S., Agrawal, L., Raj, R., Srivastava, A., Gupta, S., Mishra, S. K., Yadav, S., Singh, P. C., Raj, S. K. & Nautiyal, C. S. (2016). *Paenibacillus lentimorbus* Inoculation Enhances Tobacco Growth and Extenuates the Virulence of Cucumber mosaic virus. *PloS One*, 11(3), e0149980. https://doi.org/10.1371/journal.pone.0149980
26. Li, X., Luo, L., Yang, J., Li, B. & Yuan, H. (2015). Mechanisms for solubilization of various insoluble phosphates and activation of immobilized phosphates in different soils by an efficient and salinity-tolerant *Aspergillus niger* strain An2. *Applied Biochemistry and Biotechnology*, 175(5), 2755–2768. https://doi.org/10.1007/s12010-014-1465-2
27. Lin, L., Li, C., Ren, Z., Qin, Y., Wang, R., Wang, J., Cai, J., Zhao, L., Li, X., Cai, Y. & Xiong, X. (2023). Transcriptome profiling of genes regulated by phosphate-solubilizing bacteria *Bacillus megaterium* P68 in potato (*Solanum tuberosum* L.). *Frontiers in Microbiology*, 14, 1140752. https://doi.org/10.3389/fmicb.2023.1140752
28. Liu, Z., Li, Y. C., Zhang, S., Fu, Y., Fan, X., Patel, J. S. & Zhang, M. (2015). Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. *Applied Soil Ecology: A Section of Agriculture, Ecosystems & Environment*, 96, 217–224. https://doi.org/10.1016/j.apsoil.2015.08.003
29. Malhotra, H., Vandana, Sharma, S. & Pandey, R. (2018). Phosphorus nutrition: Plant growth in response to deficiency and excess. In: *Plant Nutrients and Abiotic Stress Tolerance* (pp. 171–190). Springer Singapore. https://doi.org/10.1007/978-981-10-9044-8_7
30. Mussarat, M., Shair, M., Muhammad, D., Mian, I. A., Khan, S., Adnan, M., Fahad, S., S. Dessoky, E., EL Sabagh, A., Zia, A., Khan, B., Shahzad, H., Anwar, S., Ilahi, H., Ahmad, M., Bibi, H., Adnan, M. & Khan, F. (2021). Accentuating the role of nitrogen to phosphorus ratio on the growth and yield of wheat crop. *Sustainability*, 13(4), 2253. https://doi.org/10.3390/su13042253
31. Nozari, R. M., Ortolan, F., Astarita, L. V., & Santarém, E. R. (2021). Streptomyces spp. enhance vegetative growth of maize plants under saline stress. *Brazilian Journal of Microbiology*, *52*(3), 1371-1383. https://doi.org/10.1007/s42770-021-00480-9
32. Raza, A., Ejaz, S., Saleem, M. S., Hejnak, V., Ahmad, F., Ahmed, M. A. A., Alotaibi, S. S., El-Shehawi, A. M., Alsubeie, M. S. & Zuan, A. T. K. (2021). Plant growth promoting rhizobacteria improve growth and yield related attributes of chili under low nitrogen availability. *PloS One*, 16(12), e0261468. https://doi.org/10.1371/journal.pone.0261468
33. Remonsellez, F., Castro-Severyn, J., Pardo-Esté, C., Aguilar, P., Fortt, J., Salinas, C., Barahona, S., León, J., Fuentes, B., Areche, C., Hernández, K. L., Aguayo, D. & Saavedra, C. P. (2018). Characterization and salt response in recurrent halotolerant *Exiguobacterium* sp. SH31 isolated from sediments of Salar de Huasco, Chilean Altiplano. *Frontiers in Microbiology*, *9*, 2228. https://doi.org/10.3389/fmicb.2018.02228
34. Sahu, A. (2014). Status of Soil in Purba Medinipur District, West Bengal–A Review. *Indian Journal of Geography and Environment*, 13,121-126.
35. Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution,* 4(4), 406-25. 10.1093/oxfordjournals.molbev.a040454
36. Sandström, J. P., Russell, J. A., White, J. P. & Moran, N. A. (2001). Independent origins and horizontal transfer of bacterial symbionts of aphids. *Molecular Ecology*, 10(1), 217–228. https://doi.org/10.1046/j.1365-294x.2001.01189.x
37. Suleman, M., Yasmin, S., Rasul, M., Yahya, M., Atta, B. M. & Mirza, M. S. (2018). Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. *PloS One*, 13(9), e0204408. https://doi.org/10.1371/journal.pone.0204408
38. Teng, Z., Chen, Z., Zhang, Q., Yao, Y., Song, M. & Li, M. (2019). Isolation and characterization of phosphate solubilizing bacteria from rhizosphere soils of the Yeyahu Wetland in Beijing, China. *Environmental Science and Pollution Research International*, 26(33), 33976–33987. https://doi.org/10.1007/s11356-018-2955-5
39. Tian, J., Ge, F., Zhang, D., Deng, S. & Liu, X. (2021). Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. *Biology*, 10(2), 158. https://doi.org/10.3390/biology10020158
40. Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991