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## Research Article

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**Metabolomic characterization and plant growth-promoting traits of *Cytobacillus oceanisediminis* PP10 isolated from the *Plantago ovata* rhizosphere**

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## Abstract

Fungal phytopathogens and soil salinity pose a severe threat to global agriculture by drastically reducing crop yield and quality. In this study, a multifunctional, halotolerant plant growth-promoting rhizobacterium (PGPR) was isolated from the rhizosphere of *Plantago ovata* and identified as *Cytobacillus oceanisediminis* strain PP10 using 16S rRNA gene sequencing. In dual culture antagonism assays, strain PP10 exhibited antifungal activity, inhibiting *Fusarium* sp. mycelial growth by 60%. Quantitative and qualitative characterization demonstrated plant growth-promoting (PGP) traits, including the production of indole-3-acetic acid (IAA), gibberellic acid (GA), ammonia, exopolysaccharides (EPS), hydrolytic enzymes, and phosphate solubilization. Strain PP10 demonstrated halotolerance, sustaining active growth at salinity levels up to 13% (w/v) NaCl. Comprehensive secretome profiling using Liquid chromatography – mass spectrometry (LC-MS) and Gas chromatography – mass spectrometry (GC-MS) identified an array of bioactive secondary metabolites and antifungal volatiles, notably pyrrolnitrin, 2,4-di-tert-butylphenol, rhamnolipids, 2-piperidinone, desferrioxamine A1, palmitic acid, and stearic acid. *In vivo* pot trials with *P. ovata* demonstrated that PP10 bacterization significantly enhanced seed germination, root/shoot vigor, and biomass accumulation. Collectively, these findings present the first report of *Cytobacillus oceanisediminis* PP10 as a novel bacterium showing plant growth promoting traits that significantly enhanced vegetative growth in *P. ovata*, demonstrating its potential as a biofertilizer for sustainable agriculture.

**Keywords:** *Cytobacillus oceanisediminis*, *Plantago ovata*, Antifungal secondary metabolites, Plant growth-promoting traits, Metabolomics

## Introduction

Global agricultural stability faces an increasing threat from pests and plant pathogens. These phytopathogens cause severe economic damage by destroying up to 40% of global crop production before harvest. Among these threats, fungal phytopathogens pose the most pervasive challenge, alone accounting for a 10 to 20% reduction in annual yields and compromising overall crop value [31]. Current crop protection approaches rely heavily on host plant resistance and chemical pesticides [6]. However, the long-term viability of these practices is hindered by volatile disease control, chemical pollution, and severe disruption of ecological equilibrium within agroecosystems.

Due to rising concerns over chemical-based agriculture, biological control provides a practical, eco-friendly alternative for effective disease management [56]. This approach uses beneficial microbes to limit the growth of pathogens and pests [14]. Differing from synthetic agrochemicals, these bio-based agents break down easily, are environmentally safe, and exhibit targeted rhizosphere activity, minimizing non-target effects [37].

Biocontrol microbes eliminate pathogens directly by producing antifungal secondary metabolites, lipopeptides, and cell-wall-degrading enzymes (such as chitinases and beta-glucanases) or indirectly by triggering systemic plant immunity [7]. At the same time, these plant growth-promoting rhizobacteria (PGPR) actively stimulate crop development by improving nutrient uptake through phosphate solubilization and ammonia production. Additionally, they synthesize regulatory phytohormones, mainly indole-3-acetic acid (IAA), gibberellic acid (GA), and cytokinins, while forming biofilms to facilitate persistent root colonization [63].

*Plantago ovata* (Isabgol), an annual herb with Persian origin, is a drought-tolerant crop grown predominantly in the semi-arid zones of western India. Belonging to the family Plantaginaceae, it is one of only two commercially grown species out of approximately 200 within the genus [25]. This lucrative, versatile plant thrives in dry conditions and is produced primarily for its fiber-rich seed husk (psyllium). Renowned for its therapeutic properties, psyllium is widely utilized across dietary, pharmaceutical, and nutraceutical industries to promote digestive health [32].

Investigating the specialized rhizosphere of *P. ovata* in harsh, semi-arid conditions presents a prime frontier for discovering biological control agents. While common genera like *Bacillus* are well documented, the functional abilities of the genus *Cytobacillus* and specifically *Cytobacillus oceanisediminis* remain largely unexplored. Significantly, past work on the *P. ovata* microbiome has been restricted to standard plant growth traits. This leaves a critical knowledge gap on indigenous, multi-functional strains that can simultaneously provide disease suppression and boost plant development.

To address this knowledge gap, the objective of the present study was to isolate and characterize a PGPR from the rhizosphere of *P. ovata* and evaluate its potential as a biocontrol and plant growth-promoting agent. The isolated strain was assessed for plant growth-promoting traits, lytic enzyme production, antifungal activity against fungal phytopathogens, and tolerance to salinity stress. In addition, liquid chromatography - mass spectrometry (LC-MS) and gas chromatography - mass spectrometry (GC-MS) analyses were performed to identify the volatile and non-volatile secondary metabolites associated with its biocontrol potential. Finally, the efficacy of the strain in promoting plant growth was evaluated through pot experiments using *P. ovata*. The findings of this study

provide insight into the potential application of the isolated strain as a plant growth-promoting and biocontrol agent for sustainable agriculture.

## **2. Materials and methods**

### **2.1 Sample collection, isolation and preliminary characterization of rhizobacteria**

Rhizospheric soil was collected from the 10-15 cm root zone of healthy *P. ovata* plants from the Surendranagar district (22.73°N, 71.62°E), Gujarat, India. Samples were preserved at 4 °C and processed within 24 hours. For the isolation of phosphate-solubilizing bacteria (PSB), 1 g of soil was serially diluted up to 10<sup>-7</sup> in sterile distilled water, and 0.1 mL was spread-plated onto Pikovskaya's (PVK) agar. Following incubation at 28 ± 2 °C for 48 hours, halocentric screening and re-streaking on PVK agar identified putative phosphate-solubilizing isolates [19]. Then the isolates were subjected to phenotypic and biochemical characterization.

### **2.2 Antagonistic Dual Culture Screening**

The *in vitro* biocontrol efficacy of strain PP10 was evaluated against *Fusarium* sp. using a dual-culture assay. A 5 mm fungal mycelial plug was placed at the center of a potato dextrose agar (PDA) plate and flanked by a parallel streak of strain PP10 at a 3 cm distance. Control plates contained only the fungal plug. Following incubation at 28 ± 2 °C for 7 days, the percentage inhibition of growth was calculated as per the following equation [4]:

$$\text{Mycelial growth inhibition (\%)} = 1 - \frac{\text{Fungal growth}}{\text{Control growth}} \times 100$$

All assays were executed in triplicates.

### **2.3 Functional Evaluation of Plant Growth-Promoting (PGP) Attributes**

### **2.3.1 IAA production assay**

IAA production was quantified according to [18]. The strain PP10 was inoculated into nutrient broth supplemented with 0.1% L-tryptophan and incubated at  $28 \pm 2$  °C under shaking conditions (150 rpm). Aliquots were collected at 24, 48, and 72 hours and centrifuged at 10,000 rpm for 10 min. Subsequently, cell-free supernatant (2 mL) was mixed with Salkowski reagent (4 mL) and incubated in the dark for 30 min at room temperature. The development of a pink color indicated IAA production, and the spectrophotometric absorbance was measured at 530 nm against a standard curve of pure IAA [57].

### **2.3.2 GA production assay**

GA production by strain PP10 was quantified as per [58]. Strain PP10 was inoculated in nutrient broth supplemented with 1mM L-tryptophan at  $27 \pm 2$  °C at 120 rpm, with samples harvested at 24, 48, and 72 hours. Supernatant (1 mL) recovered by centrifugation (5000 rpm, 20 min) was mixed with Folin–Ciocalteu reagent (1 mL) and concentrated HCl (1 mL), boiled for 5 min, and cooled. The greenish-blue chromophore was measured at 760 nm against a standard GA curve [2].

### **2.3.3 Phosphate solubilization assay**

Qualitative phosphate solubilization was assessed by spot inoculating strain PP10 onto PVK agar amended with  $\text{Ca}_3(\text{PO}_4)_2$  as an insoluble tricalcium phosphate source. After incubation at  $27 \pm 3$  °C for 7 days, the phosphate solubilization index (PSI) and phosphate solubilization efficiency (PSE) was calculated as follows:

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

$$PSE (\%) = \frac{\text{Halozone diameter}}{\text{Colony diameter}} \times 100$$

For quantification, strain PP10 was inoculated in PVK broth at  $27 \pm 3$  °C and 120 rpm. On days 3, 5, and 7, culture aliquots were collected and centrifuged at 5000 rpm for 20 min. Solubilized phosphate in the cell-free supernatant was quantified at 600 nm using the stannous chloride method against a  $\text{KH}_2\text{PO}_4$  standard curve. Uninoculated PVK broth served as the control [42].

#### **2.3.4 Ammonia production assay**

Strain PP10 was inoculated into peptone water at  $27 \pm 3$  °C and 120 rpm. Aliquots (1 mL) were aseptically withdrawn at 24, 48, and 72 hours and centrifuged at 5000 rpm for 20 min. The resulting supernatant (0.2 mL) was reacted with 1 mL of Nessler's reagent, and yellow-to-brown colour development was resolved spectrophotometrically at 425 nm against an uninoculated blank, with concentrations calculated using an ammonium sulphate reference curve [29].

#### **2.4 *In vitro* assessment of EPS production: Qualitative and Quantitative**

EPS secretion by strain PP10 was screened on Congo red agar (CRA) medium supplemented with sucrose. Following incubation at  $27 \pm 3$  °C for 48 hours, positive production was confirmed by characteristic black, mucoid colonies [17].

Biofilm formation was quantified using the tissue culture plate (TCP) assay [11]. Strain PP10 was inoculated in trypticase soy broth (TSB) at 37 °C for 24 hours, diluted 1:100 in

fresh medium, and inoculated 0.2 mL into polystyrene microplates. After incubation at 37 °C for 24 hours, non-adherent planktonic cells were removed by washing twice with phosphate-buffered saline (pH 7.2). The adherent biofilm matrix was heat-fixed at 37 °C for 1 hour, stained with 0.2 mL of 0.1% crystal violet for 10 minutes, and rinsed with deionized water. Bound dye was solubilized using 200 µL of 33% glacial acetic acid, and optical density was measured at 570 nm using a Bio-Rad 680 microplate reader. TSB served as the control, and traits were stratified into strong, moderate, or weak phenotypes.

## **2.5 Halotolerance Assessment of strain PP10**

Strain PP10 was inoculated in nutrient broth supplemented with varying NaCl concentrations (1%, 3%, 5%, 7%, 9%, 11%, and 13%) and incubated at  $28 \pm 2$  °C with shaking. Growth kinetics were monitored at 24, 48, and 72 hours by measuring optical density at 600 nm. Assays were executed in triplicates, and the data were expressed as mean  $\pm$  standard deviation.

## **2.6 Hydrolytic Enzyme Assays**

### **2.6.1 Protease activity: Screening and Quantification**

Qualitative proteolytic potential of strain PP10 was screened on skimmed milk agar plates at  $30 \pm 2$  °C for 72 hours; positive activity was confirmed by the development of clear halo zones around the colonies [36].

For quantitative analysis, the crude enzyme was harvested at 24, 48, and 72 hours by centrifugation at 8,000 rpm for 20 min at 4 °C from the culture grown in protease production broth at 30 °C and 120 rpm. The assay mixture containing 1 mL supernatant

and 2 mL of casein in 50 mM phosphate buffer (pH 7.0) was incubated at room temperature for 40 min; proteolysis was terminated by adding 5% trichloroacetic acid (TCA) and allowed to stand for 30 minutes. Liberated tyrosine in the supernatant was quantified at 660 nm [15].

### **2.6.2 Lipase Activity: Screening and Quantification**

Lipolytic screening of strain PP10 was performed on nutrient agar supplemented with egg yolk at  $30 \pm 2$  °C for 48 hours, where positive lipolysis was identified by the formation of clear or opalescent halo zones surrounding the colonies [16].

Lipase activity was quantified titrimetrically via substrate-specific olive oil hydrolysis. Crude enzyme was harvested from culture aliquots at 24, 48, and 72 hours via centrifugation at 8,000 rpm for 20 min. We then incubated a mixture of 1 mL supernatant, 1 mL of 0.1 M Tris–HCl buffer (pH 8.0), 2.5 mL deionized water, and 3 mL olive oil at 37 °C for 30 minutes. Substrate cleavage was terminated with 2 mL chilled acetone. After stopping the reaction with 2 mL chilled acetone, the released free fatty acids were titrated against 0.1 M NaOH with a phenolphthalein indicator until a persistent pink endpoint was reached [5, 47].

### **2.7 16S rRNA Gene Sequencing and Phylogenetic Architecture**

Genomic DNA was extracted from the strain PP10 and evaluated for integrity on a 1% agarose gel. The near full-length 16S rRNA gene was selectively amplified via PCR using universal primers (27F/1391R), column-purified, and sequenced on an ABI 3500xL Genetic Analyzer using BigDye Terminator v3.1 chemistry. The resulting consensus sequence was deposited in the NCBI GenBank database under an accession number

PX020695. Homologous reference sequences were retrieved from GenBank for multiple sequence alignment, and a phylogenetic tree was constructed using the neighbor-joining evolutionary method in MEGA v12 software.

## **2.8 Extraction of Bioactive Metabolites**

Secondary metabolites from strain PP10 were extracted via modified acid precipitation [4]. After cultivation in nutrient broth for at 30°C and 150 rpm for 96 hours. The biomass was cleared via centrifugation at 10,000 rpm for 10 min, and the supernatant was acidified to pH 2.0 (1M HCl) and precipitated overnight at 4 °C. The crude fraction was pelleted (10,000 rpm) and dissolved in methanol, then passed through a 0.22 µm syringe filter. The methanolic extract was then analyzed on an Agilent LC–MS system with a reverse-phase C<sub>18</sub> column using a 15 min linear gradient of mobile phase A (0.1 % formic acid in H<sub>2</sub>O) and mobile phase B (0.1 % formic acid in acetonitrile) from 70:30 to 10:90 (A: B) at 0.3 mL/min, with a sample injection of 20 µl. Mass spectra were acquired through a positive electrospray ionization mode across an m/z range of 45-1700, and metabolites were tentatively identified by comparing retention time and m/z values against spectral databases and literature.

## **2.9 Extraction of volatile metabolites**

Volatile metabolites from strain PP10 were extracted as described by [54] with minor modifications. The strain PP10 was cultivated in 100 mL of Nutrient broth at 28 ± 2 °C for 72 hours (150 rpm). After centrifugation at 10,000 rpm for 15 minutes, the supernatant was acidified to pH 2.0 under agitation for 30 minutes. Extracellular fractions were partitioned thrice by adding an equal volume of ethyl acetate and shaking for 2 hours. The

organic phase was resolved in a separating funnel. The pooled fraction was concentrated under vacuum evaporation at 35 °C, and the resulting dried crude extract was stored at -20 °C. Before analysis, the dried crude extract was silylated using N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS).

Then analyzed on a Clarus 500 GC–MS system (PerkinElmer, USA) using helium carrier gas (1 mL/min, 99.999% purity). Injector and ion source temperatures were maintained at 250 °C and 200 °C, respectively. Electron ionization (70 eV) was operated across an m/z range of 45 - 600 following a 2 min solvent delay. The oven thermal profile started at 100 °C, ramped at 10 °C/min to 200 °C, and finally increased at 5 °C/min to a terminal hold at 280 °C. Volatiles were identified by matching mass spectra against the NIST reference library.

## **2.10 In Planta Growth Promotion Study**

The plant growth–promoting efficacy of strain PP10 was evaluated on *P. ovata* under greenhouse pot conditions. Viable seeds were surface-sterilized with 0.2% sodium hypochlorite for 2 minutes, rinsed 7 times in sterile distilled water, coated with 1% carboxymethyl cellulose adhesive filler, and immersed in the bacterial suspension for 30 minutes. Inoculum were prepared by cultivating strain PP10 in nutrient broth at  $28 \pm 2$  °C for 48 hours to log-phase densities. Plastic pots pre-filled with sterile soil were sown with five treated seeds each, alongside non-inoculated controls using a completely randomized design with three biological replicates [25]. Seed germination was monitored daily to calculate emergence. Growth parameters including germination percentage, root

length, shoot length, total plant length, vigor index, and chlorophyll content were recorded at 35 and 70 days after sowing (DAS) to assess the effect of the bacterial isolate on plant growth.

#### **2.10.1 Chlorophyll content estimation**

Chlorophyll content was determined by homogenizing 0.5 g of fresh leaf tissue in 80% acetone [20]. The centrifuged supernatant was measured at 645 and 663 nm, and the levels of total chlorophyll, chlorophyll a, and chlorophyll b were computed using Arnon's equations and expressed as mg/g fresh weight.

#### **2.11 Statistical analysis**

All experiments were performed in triplicate with results expressed as mean  $\pm$  standard deviation (SD). The data from the pot trials were analyzed using one-way analysis of variance (ANOVA) to evaluate the significance of differences between treatments and the control. Statistical significance was determined at  $p < 0.05$ .

### 3 Results

#### 3.1 Isolation and polyphasic characterization of strain PP10 from the rhizosphere of *Plantago ovata*

Rhizospheric soil sample of *P. ovata* yielded 40 bacterial isolates. Among these, strain PP10 exhibited the highest potential during preliminary screening and was therefore selected for further functional characterization. Strain PP10 produced creamy white colonies with a smooth margin and convex elevation when grown on nutrient agar. Microscopic examination revealed that the isolate was Gram-positive, rod-shaped, and encapsulated bacterium (Fig. 1).

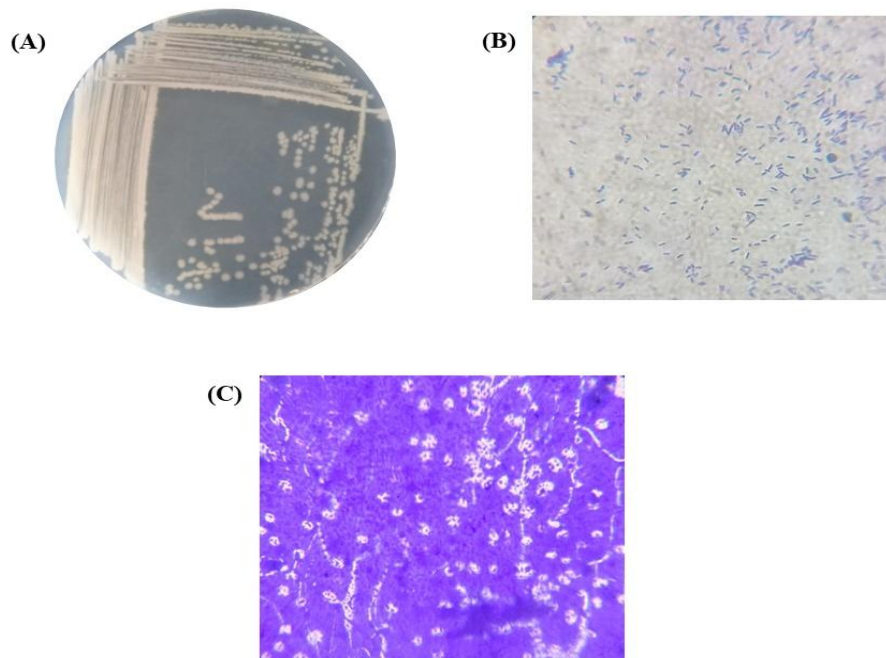


**Fig. 1 Morphological profiling of strain PP10: (A) Colony morphology on nutrient agar plate; (B) Microscopic image showing Gram-positive reaction; (C) Microscopic detection of capsule formation**

Biochemical characterization showed that strain PP10 was positive for citrate utilization, gelatin hydrolysis, motility, and oxidative enzymes, including catalase, oxidase, and dehydrogenase. In contrast, negative results were obtained for urease, methyl red (MR), Voges-Proskauer (VP), indole, phenylalanine deaminase, and hydrogen sulfide formation.

Carbohydrate utilization assays showed selective fermentation of glucose, fructose, sucrose, maltose, and mannitol, while lactose and sorbitol were not fermented, with no gas production observed.

### 3.2 Assessment of Antagonistic activity by Dual Culture Assay

Strain PP10 displayed pronounced antagonism against *Fusarium* sp. in dual culture, with a 60% reduction in fungal mycelial growth and restricted hyphal advancement compared to the control (Fig. 2).

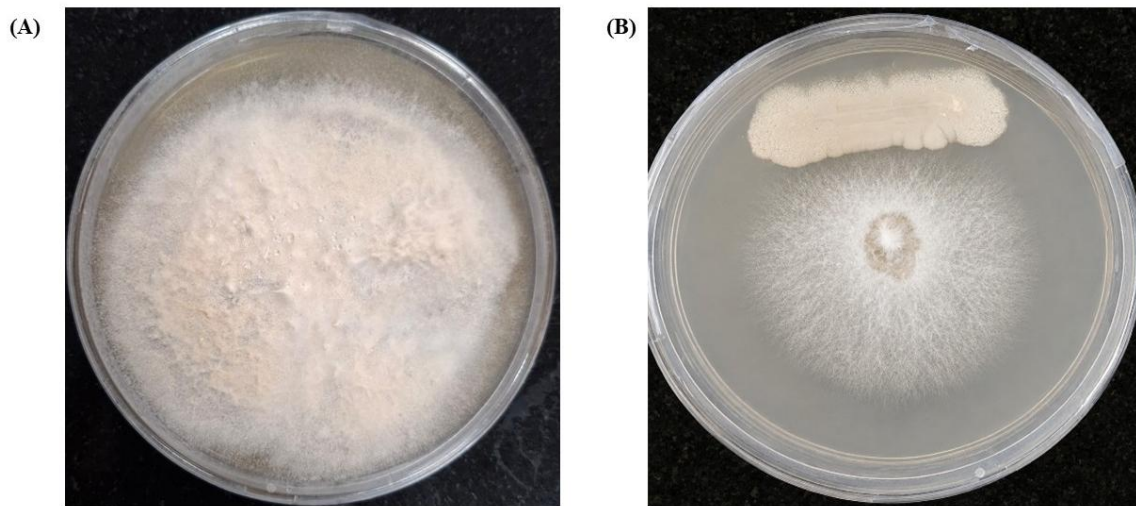


**Fig. 2** *In vitro* biocontrol efficacy of strain PP10 against *Fusarium* sp. (A) control plate showing unrestricted fungal growth; (B) Dual culture plate showing restricted fungal mycelial growth by strain PP10

### 3.3 Plant growth promoting attributes of PP10

#### 3.3.1 IAA production

We initially detected IAA production by strain PP10 through the appearance of a characteristic pink color upon the addition of Salkowski reagent as shown in (Fig. 3A). Quantitative estimation based on the IAA standard calibration curve revealed a time-dependent increase in IAA production, with concentrations of 68, 99, and 130  $\mu\text{g/mL}$  recorded after 24, 48 and 72 hours of incubation, respectively (Fig. 3B).

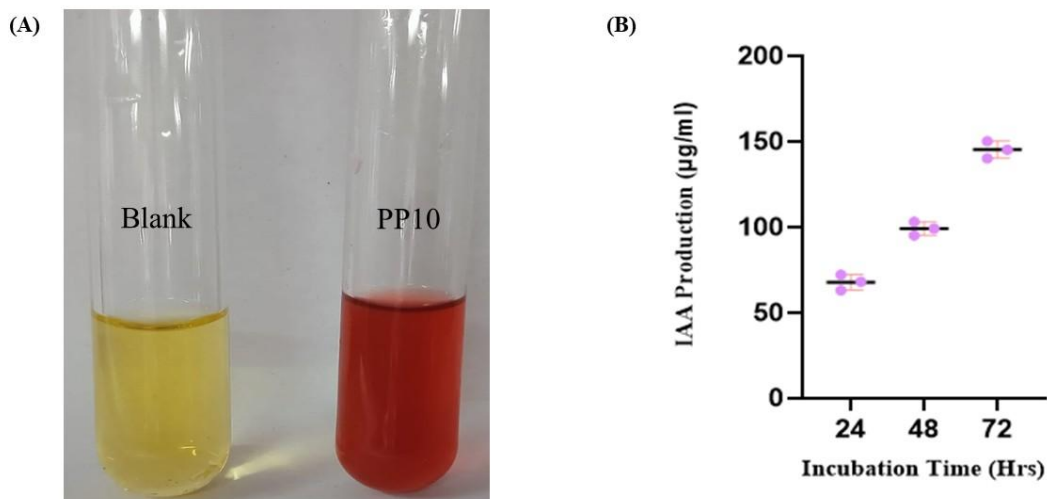


**Fig. 3** Indole-3-acetic acid production by strain PP10. (A) Visual confirmation of IAA production compared to blank control; (B) Time course quantification of IAA yield

over a 72-hour incubation period. Data points represent independent replicates, and horizontal bars indicate the mean values ( $\pm$ SD)

### 3.3.2 GA production

GA production by the strain PP10 was evaluated using a colorimetric assay, confirming its ability to synthesize gibberellin-like compounds. Quantitative estimation revealed that strain PP10 produced 66  $\mu$ g/mL after 24 h of incubation, which increased to 89  $\mu$ g/mL at 48 h, with the maximum gibberellin production of 105  $\mu$ g/mL recorded after 72 h of incubation (Fig. 4).

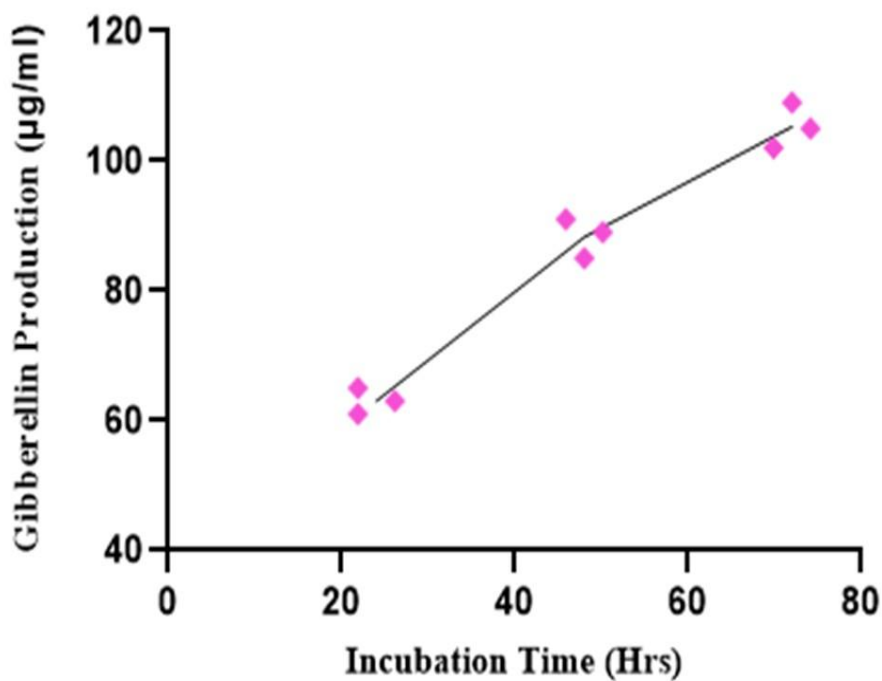


Fig. 4 Quantitative evaluation of GA production by strain PP10 over a 72-hour incubation period. Data points represent individual replicates.

### 3.3.3 Phosphate solubilization

Phosphate solubilization by the strain PP10 was qualitatively confirmed by the formation of a clear halo zone around the bacterial colonies on Pikovskaya's agar medium, as shown in Fig. 5A. The solubilization index (SI) and solubilization efficiency (SE) recorded values of 4.9 and 390% after 7 days of incubation (Fig. 5B).

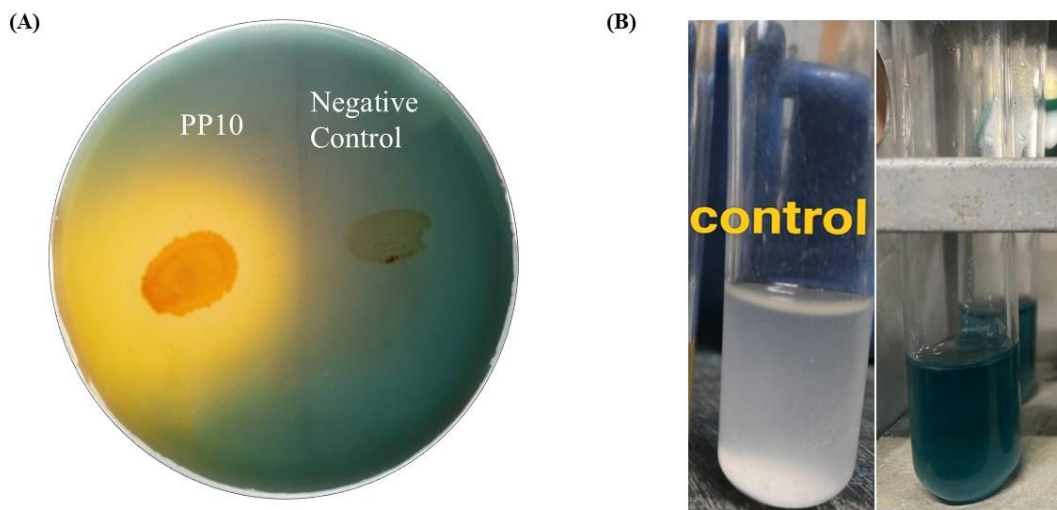


**Fig. 5 (A) Pikovskaya's agar plate assay demonstrating a clear zone around strain PP10 as compared to the negative control; (B) liquid assay showing color change as compared to the blank**

Strain PP10 exhibited time dependent phosphate solubilization in liquid PVK medium with the concentration of 35  $\mu\text{g/mL}$  on the 3rd day to 56  $\mu\text{g/mL}$  on the 5th day reaching a maximum of 118  $\mu\text{g/mL}$  after 7 days of incubation, which was concurred with a gradual decline in the culture pH from 10 to 8 and 5, respectively (Fig. 6). This increase in soluble phosphate was accompanied by a gradual decline in medium pH, suggesting organic acid-mediated phosphate solubilization.

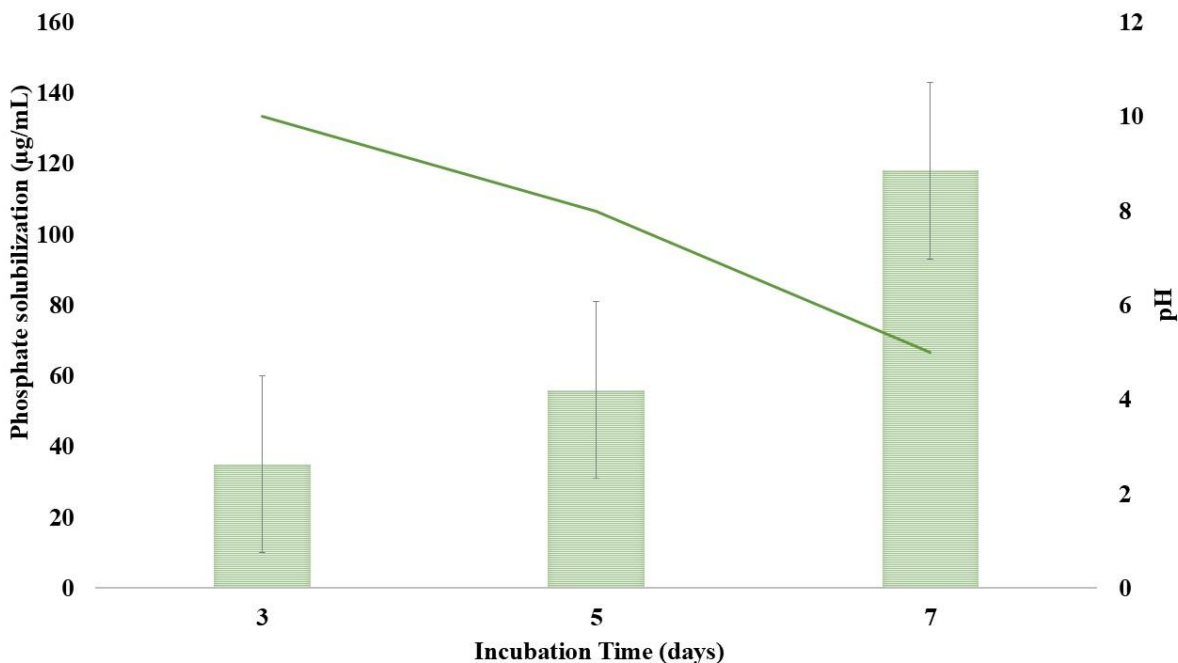


**Fig. 6 Time course evaluation of phosphate solubilization efficiency and pH reduction by strain PP10. Error bars denote the standard deviation.**

### 3.3.4 Ammonia production

Ammonia production by strain PP10 was qualitatively indicated by the development of a distinct yellow to brown coloration in the culture supernatant, as shown in Fig. 7A.

Quantitative analysis in peptone water revealed time-dependent accumulation of ammonia rising from 56 µg/mL at 24 hours to 90 µg/mL at 48 hours, with the maximum ammonia production of 120 µg/mL recorded after 72 hours of incubation (Fig. 7B). This progressive increase reflects sustained nitrogen mineralization activity by the isolate.

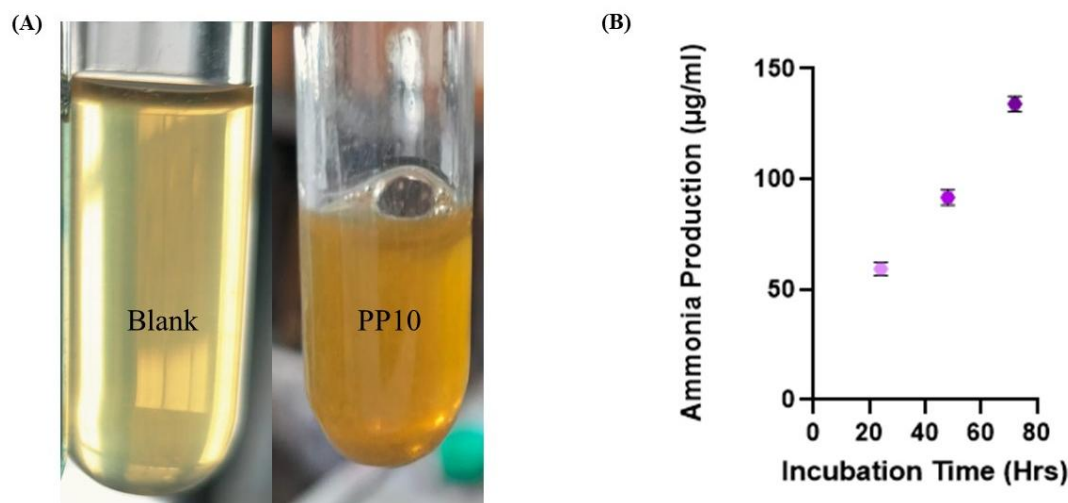


**Fig. 7 Ammonia production by strain PP10 (A) Qualitative screening showing color change as compared to blank; (B) Quantitative evaluation over 72-hour incubation period. Data points represent individual replicates, with error bars denoting mean  $\pm$  SD.**

### **3.4 EPS production**

Strain PP10 demonstrated the ability to produce EPS, as observed in both qualitative and quantitative assays. Qualitative assessment on congo red agar revealed the formation of mucoid, darkly stained colonies, indicating robust EPS secretion (Fig. 8A).

For quantitative evaluation, EPS production was measured using the TCP assay (Fig. 8B).

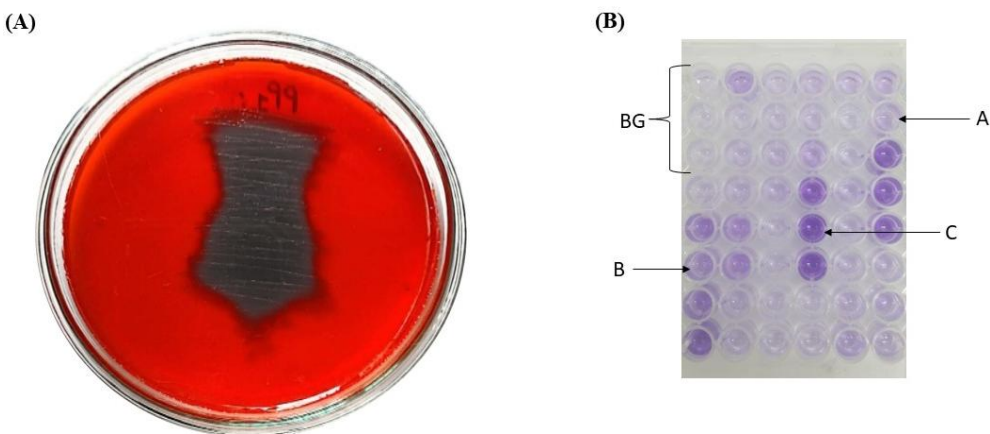


**Fig. 8 Qualitative and quantitative evaluation of biofilm production by strain PP10: (A) Congo red agar plate assay showing black-colored colonies indicating EPS production by strain PP10; (B) Microtiter plate assay stained with crystal violet for biofilm quantification illustrating the background wells (BG) alongside sample wells (A: weak, B: moderate, C: strong biofilm formation).**

### **3.5 Effect of salinity stress on the growth of PP10**

The salt tolerance ability of strain PP10 was evaluated across a range of NaCl concentrations, and the isolate exhibited sustained growth even at 13% NaCl (Fig. 9).

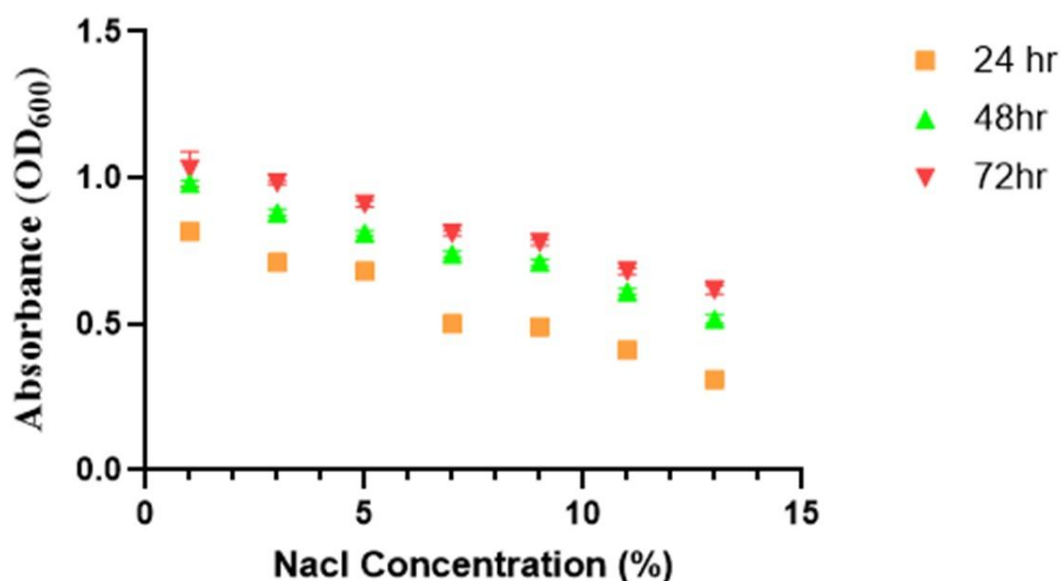


**Fig. 9 Salinity tolerance of strain PP10 under varying NaCl concentrations. Absorbance (OD<sub>600</sub>) was tracked over 72 hours of incubation. Error bars represent the mean  $\pm$  SD of the experiment**

### **3.6 Screening of hydrolytic enzymes in strain PP10**

#### **3.6.1 Protease Activity**

Protease production by strain PP10 was qualitatively confirmed by a clear halo zone around the colonies on skimmed milk agar (Fig. 10A). Quantitative evaluation showed a progressive increase in enzyme activity from 1.6 U/mL at 24 h to 3.5 U/mL at 48 h and peaking at 6 U/mL at 72 h, respectively (Fig. 10B).



**Fig. 10** Proteolytic assay by strain PP10: (A) Skimmed milk agar plate assay showing clear zones of hydrolysis around the isolate PP10; (B) Quantification of protease activity over a 72-hour incubation period. Error bars denote the mean  $\pm$  SD.

### 3.6.2 Lipase Activity

Qualitative screening on egg yolk agar medium confirmed extracellular lipase secretion by forming a halo zone around the colonies (Fig. 11A). Quantitative lipase production was recorded as 6 U/mL at 24 h to 9.3 U/mL at 48 h, and attained the highest level of 11.5 U/mL at 72 h (Fig. 11B).

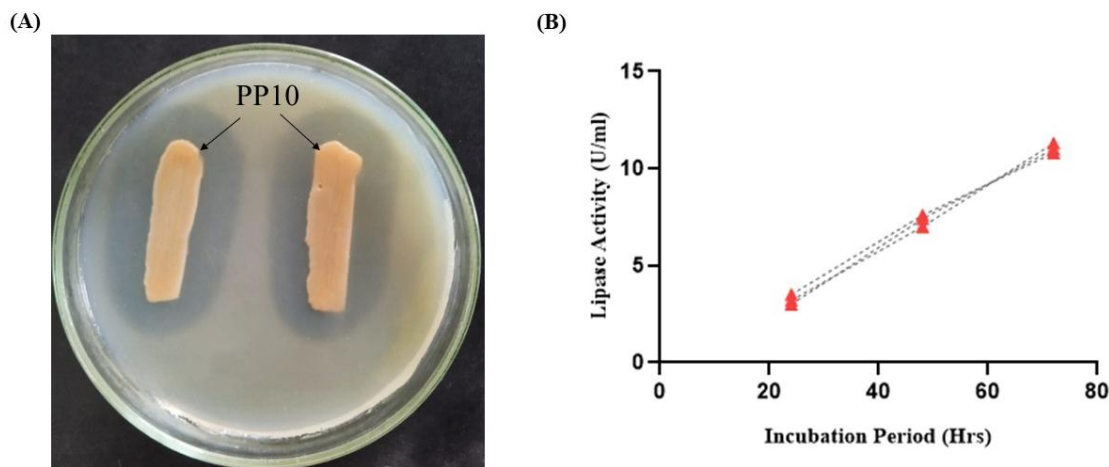


**Fig. 11 Lipase activity by strain PP10 (A) Egg yolk agar plate showing zone of clearance by strain PP10; (B) Quantitative estimation over 72-hour incubation period by strain PP10.**

### **3.7 Molecular identification of strain PP10**

The evolutionary relationships of strain PP10 were inferred using the neighbour joining method with bootstrap analysis based on 1000 replications. Evolutionary distances were calculated using the Jukes–Cantor substitution model, and phylogenetic analyses were performed in MEGA version 11.

Phylogenetic analysis further supported the placement of strain PP10 within the genus *Cytobacillus*, and the isolate was identified as *Cytobacillus oceanisediminis*. The sequence was deposited in the GenBank database under accession number **PX020965** (Fig. 12).

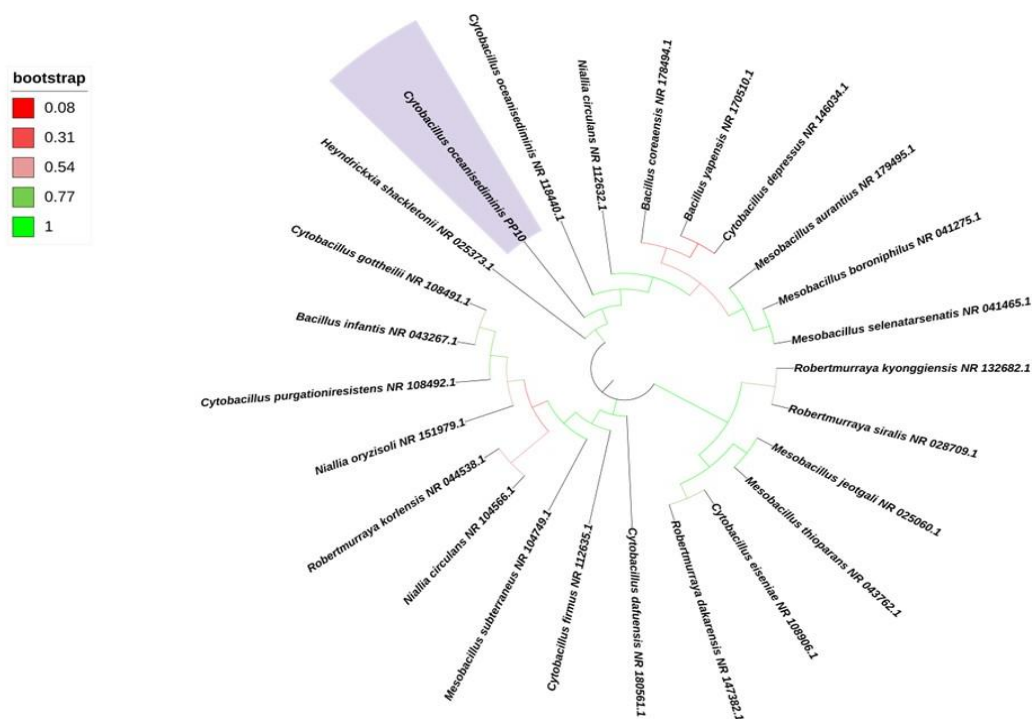


**Fig. 12 Neighbour joining phylogenetic tree of *Cytopacillus oceanisediminis* PP10.**

### **3.8 LC-MS profiling of secondary metabolites produced by isolate PP10**

LC–MS analysis of the crude extract revealed a diverse range of secondary metabolites, among which several compounds with known antifungal activity were identified (Fig. 13). Phenolic compounds such as 2,4-di-tert-butylphenol ( $m/z$  206.17) and trans-ferulic acid ( $m/z$  177.05) were identified. Quinolone derivatives including 2-heptylquinolin-4(1H)-one ( $m/z$  244.17) and 2-nonyl-3-hydroxyquinolone ( $m/z$  288.19) were also detected. A prominent peak at  $m/z$  256.98 corresponded to pyrrolnitrin, while rhamnolipid ( $m/z$  701.39) and desferrioxamine A1 ( $m/z$  547.33) were observed as higher molecular weight bioactive compounds (Table 1).

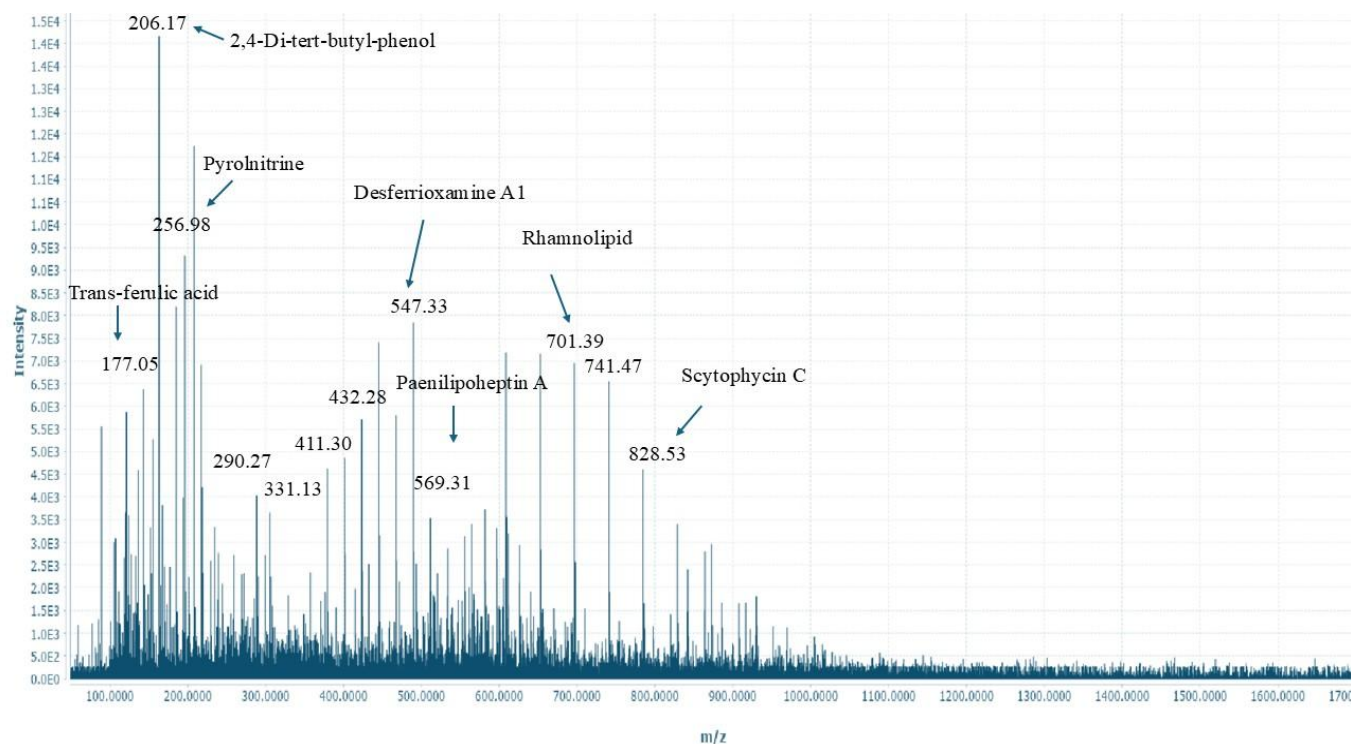


**Fig. 13 LC–ESI–MS chromatogram of methanolic extract of isolate PP10**

**Table 1 LC–MS profiling of bioactive compounds produced by isolate PP10**

| No. | Retention time | <i>m/z</i> | Molecular Formula                                             | Putative Compound                                          |
|-----|----------------|------------|---------------------------------------------------------------|------------------------------------------------------------|
| 1   | 7.87           | 206.17467  | C <sub>14</sub> H <sub>22</sub> O                             | 2,4-Di-tert-butyl-phenol                                   |
| 2   | 5.432          | 828.53072  | C <sub>45</sub> H <sub>75</sub> NO <sub>11</sub>              | Scytophycin C                                              |
| 3   | 7.836          | 206.15812  | C <sub>8</sub> H <sub>12</sub> CIN                            | Phenylethylamide 205                                       |
| 4   | 5.344          | 741.47478  | C <sub>41</sub> H <sub>64</sub> N <sub>4</sub> O <sub>8</sub> | Antanapeptin C                                             |
| 5   | 5.248          | 653.42067  | C <sub>20</sub> H <sub>29</sub> NO <sub>2</sub>               | (2E,4E)-N-[2-(4-hydroxyphenyl) ethyl] dodeca-2,4-dienamide |
| 6   | 7.657          | 290.27076  | C <sub>15</sub> H <sub>14</sub> O                             | Catechin                                                   |

|    |        |           |              |                                                |
|----|--------|-----------|--------------|------------------------------------------------|
| 7  | 4.948  | 432.28131 | C27H39NO2    | Veratramine                                    |
| 8  | 8.816  | 177.05494 | C10H10O4     | Trans-Ferulic acid                             |
| 9  | 5.657  | 244.17123 | C16H21NO     | 2-heptylquinolin-4(1H)-one                     |
| 10 | 5.303  | 701.39424 | C32H58O13    | Rhamnolipid                                    |
| 11 | 5.148  | 547.33226 | C24H46N6O8   | Desferrioxamine A1                             |
| 12 | 5.144  | 569.31391 | C59H82N10O12 | Paenilipoheptin A                              |
| 13 | 5.457  | 650.3994  | C43H55NO5    | Dehydroxy Nodulisporic Acid B                  |
| 14 | 5.082  | 525.28816 | C32H36N4O3   | Okaramine J                                    |
| 15 | 8.924  | 331.13059 | C20H20O3     | Biflorin                                       |
| 16 | 7.887  | 411.30073 | C21H38N4O4   | Xenotetrapeptide                               |
| 17 | 5.757  | 320.22378 | C17H21NO5    | Dihydropiartine                                |
| 18 | 4.973  | 261.12276 | C14H16N2O3   | Pyrrolo[1,2-a]pyrazine-1,4-dione<br>derivative |
| 19 | 5.198  | 288.19577 | C18H25NO2    | 2-nonyl-3-hydroxy 4(1H)-quinolone              |
| 20 | 5.753  | 643.41081 | C36H60O8     | Ginsenoside Rh4                                |
| 21 | 2.006  | 155.08129 | C7H10N2O2    | Cyclo-prolylglycine                            |
| 22 | 5.557  | 498.3133  | C26H40O8     | Neoandrographolide                             |
| 23 | 10.675 | 281.21225 | C17H28O3     | Photopyrone C                                  |
| 24 | 1.322  | 256.9813  | C10H6Cl2N2O2 | Pyrolnitrine                                   |
| 25 | 2.452  | 213.07252 | C10H12O5     | Propyl gallate                                 |
| 26 | 8.953  | 344.31645 | C18H16O7     | Trypacidin                                     |
| 27 | 1.873  | 172.16868 | C10H18O      | NEROL                                          |
| 28 | 7.307  | 304.26365 | C20H33NO     | Fenpropimorph                                  |

|    |       |           |               |                         |
|----|-------|-----------|---------------|-------------------------|
| 29 | 5.828 | 731.46475 | C40H68O10     | Grisorixin              |
| 30 | 2.194 | 308.12065 | C17H19NO3     | Piperine                |
| 31 | 5.628 | 826.50473 | C42H69N5O10   | Viequeamide A           |
| 32 | 1.948 | 553.25992 | C29H42Cl2N2O4 | Emetine Dihydrochloride |
| 33 | 4.898 | 303.13295 | C16H18N2O4    | Tomaymycin              |
| 34 | 5.198 | 629.31326 | C38H44O8      | Gambogic acid           |
| 35 | 1.377 | 424.89615 | C27H38O4      | Stypoltrione            |

### 3.9 Characterization of volatile metabolites from strain PP10

The GC-MS analysis of the crude extract of *Cytobacillus oceanisediminis* PP10 revealed the production of multiple bioactive compounds. The chromatogram exhibited well-resolved peaks corresponding to compounds identified through comparison with standard mass spectral libraries, confirming the occurrence of fatty acids, hydrocarbons, and heterocyclic derivatives. The metabolite extract was subjected to GC-MS, and compound identification was confirmed using the NIST Mass Spectral Library (2014).

Prominent compounds were detected, such as palmitic acid (hexadecanoic acid), cis-13-eicosenoic acid, Stearic acid, 2-Piperidinone, 1-Bromo-8-heptadecyne, and 9-hexylheptadecane (Fig.14; Table 2)

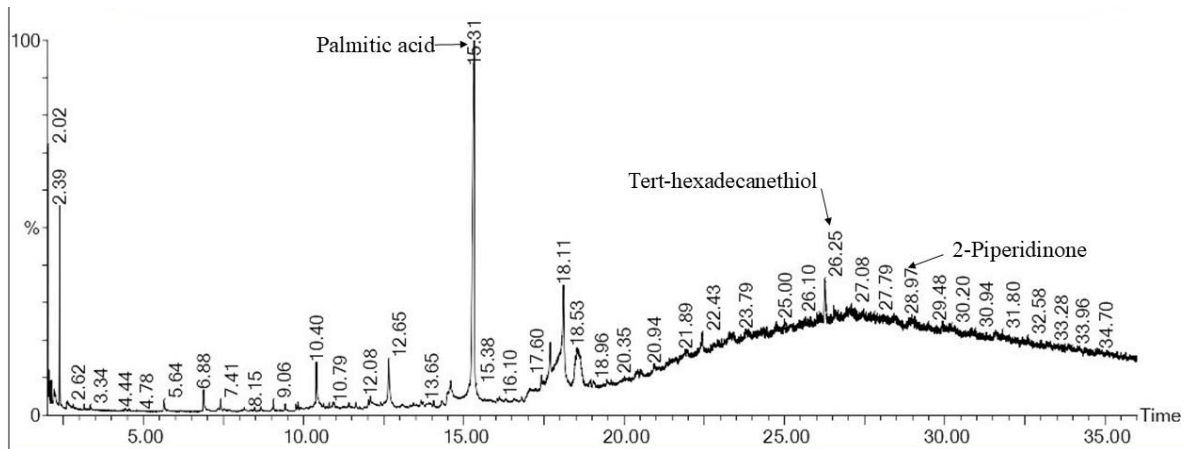


**Fig. 14 GC-MS chromatogram of volatile compounds from *Cytophilus oceanisediminis* PP10**

**Table 2 Volatile compounds identified from strain PP10**

| No | Compound Name          | Molecular Formula                              | Molecular Wt. (g/mol) | Retention Time (min) |
|----|------------------------|------------------------------------------------|-----------------------|----------------------|
| 1  | Palmitic acid          | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> | 256.42                | 15.31                |
| 2  | Stearic acid           | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> | 284.5                 | 18.11                |
| 3  | Cis-13-eicosenoic acid | C <sub>21</sub> H <sub>40</sub> O <sub>2</sub> | 324.5                 | 25.81                |
| 4  | Tert-hexadecanethiol   | C <sub>16</sub> H <sub>34</sub> S              | 258.5                 | 26.53                |
| 5  | 2-Piperidinone         | C <sub>5</sub> H <sub>9</sub> NO               | 99.13                 | 27.08                |
| 6  | 1-Bromo-8-heptadecyne  | C <sub>17</sub> H <sub>31</sub> Br             | 315.33                | 27.79                |

|   |                        |        |        |       |
|---|------------------------|--------|--------|-------|
| 7 | 9-<br>Hexylheptadecane | C23H48 | 324.38 | 28.41 |
|---|------------------------|--------|--------|-------|

### 3.10 Pot study

To elucidate the growth-promoting potential of rhizobacterial strain PP10, a pot experiment was conducted on *P. ovata* under controlled conditions at different growth stages (35 & 70 DAS). A significant enhancement in plant growth was observed in PP10-treated plants compared to the uninoculated control, as depicted in Fig. 15, whereas detailed quantitative data are presented in Table 3.

Root growth of *P. ovata* was markedly enhanced following inoculation with *Cytobacillus oceanisediminis*, with root length increasing from 7.03 cm at 35 DAS to 14.63 cm at 70 DAS compared to the uninoculated control. A similar trend was observed for root biomass, with root fresh and dry weights ranging from 0.085 to 0.176 g and 0.0080 to 0.0243 g, respectively, in PP10-treated plants compared to the uninoculated control.

Inoculation with *C. oceanisediminis* resulted in a significant increase in shoot growth of *P. ovata*, with shoot length increasing from 9.03 cm at 35 DAS to 16.43 cm at 70 DAS. Similarly, shoot fresh weight increased from 0.20 to 0.50 g, while shoot dry weight increased from 0.042 to 0.100 g under PP10 treatment in comparison to the control plants. Total chlorophyll content of *P. ovata* showed a significant increase under PP10 treatment, with values rising from 1.15 mg/g FW at 35 DAS to 2.22 mg/g FW at 70 DAS compared to the control (Table 4).

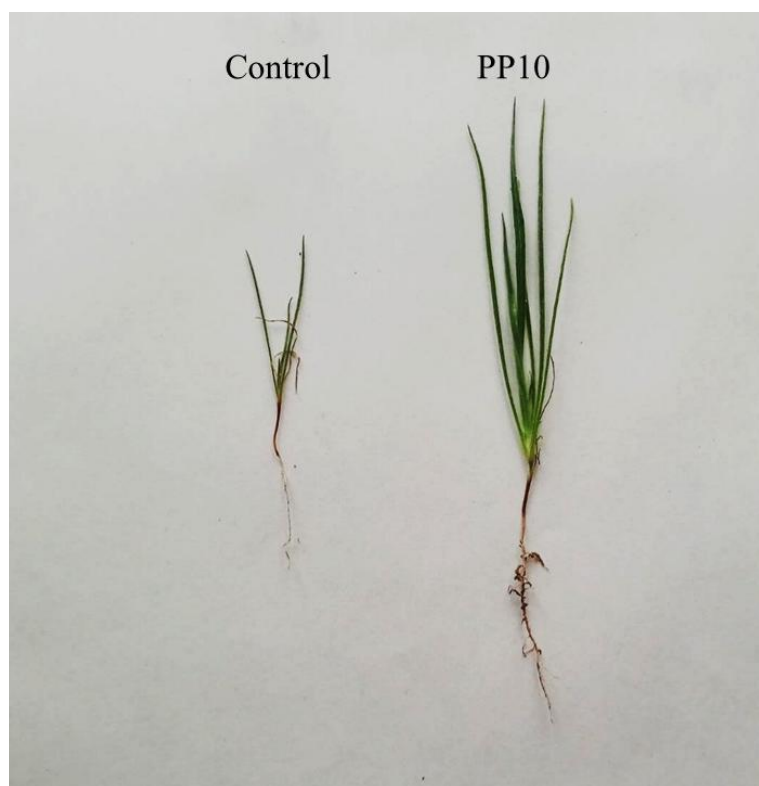


**Fig. 15 Comparative growth response of *Plantago ovata* under control and PP10 treatment**

**Table 3 Influence of *Cytobacillus oceanisediminis* PP10 on plant biometric parameters up to 70 DAS**

| Vegetative Parameters | 35 DAS        |                | 70 DAS         |                |
|-----------------------|---------------|----------------|----------------|----------------|
|                       | Control       | PP10           | C              | PP10           |
| Root length (cm)      | 4.07 ± 0.2a   | 7.03 ± 0.25b   | 8.10 ± 0.10c   | 14.63 ± 1.05d  |
| Fresh wt. (g)         | 0.02 ± 0.005a | 0.085 ± 0.005b | 0.090 ± 0.010b | 0.176 ± 0.025c |
| Dry wt. (g)           | 0.002 ± 0.00a | 0.01 ± 0.02b   | 0.008 ± 0.001b | 0.02 ± 0.004d  |
| Shoot length (cm)     | 5.1 ± 0.1 a   | 9.03 ± 0.35 b  | 9.7 ± 0.7 b    | 16.43 ± 1.02c  |

|               |                |                |                |               |
|---------------|----------------|----------------|----------------|---------------|
| Fresh wt. (g) | 0.09 ± 0.01 a  | 0.2 ± 0.01 b   | 0.18 ± 0.02 b  | 0.5 ± 0.02c   |
| Dry wt. (g)   | 0.01 ± 0.002 a | 0.04 ± 0.003 c | 0.03 ± 0.004 b | 0.1 ± 0.004 d |

DAS- days after sowing

Values represented by different lowercase letters within a column differ significantly at  $p < 0.05$  as analyzed by ANOVA.

**TABLE 4 Influence of *Cytobacillus oceanisediminis* PP10 on chlorophyll content of *Plantago ovata* up to 70 DAS**

| Chlorophyll          | 35 DAS        |               | 70 DAS        |               |
|----------------------|---------------|---------------|---------------|---------------|
|                      | Control       | PP10          | Control       | PP10          |
| Chlorophyll<br>a     | 0.34 ± 0.015a | 0.52 ± 0.025b | 0.59 ± 0.015c | 1.14 ± 0.015d |
| Chlorophyll<br>b     | 0.43 ± 0.02a  | 0.63 ± 0.01c  | 0.60 ± 0.015b | 1.08 ± 0.015d |
| Total<br>Chlorophyll | 0.77 ± 0.035a | 1.15 ± 0.035b | 1.19 ± 0.03b  | 2.22 ± 0.03c  |

Values represented by different lowercase letters within a column differ significantly at  $p < 0.05$  as analyzed by ANOVA.

## 4 Discussion

Rhizobacteria exhibiting biocontrol potential are increasingly being recognized as promising eco-friendly alternatives for sustainable plant disease management. Among these, *Cytobacillus* species have gained significant attention due to their metabolic versatility and environmental resilience [52]. Although medicinal plant rhizospheres serve as a rich reservoir for functional microbes, research on the beneficial microflora of *P. ovata* remains scarce [46]. Because the biocontrol potential of *P. ovata* associated with *Cytobacillus oceanisediminis* has not yet been documented, this study was designed to explore its novel biocontrol dynamics for sustainable agriculture.

The phenotypic characteristics of strain PP10, including Gram-positive nature, motility, and capsule formation, indicated its adaptation to rhizospheric conditions. Its peptidoglycan layer provides stress resilience, while motility guides migration towards root exudates for colonization. Furthermore, capsule formation aids in bacterial attachment, persistence, and survival on root surfaces [9, 60].

Plants continuously face growth limiting environmental and oxidative stresses which are mitigated by beneficial rhizosphere microbes through protective enzymes and metabolites [3, 10]. Mechanistically, strain PP10 showed positive catalase activity for reactive oxygen species detoxification, while oxidase and dehydrogenase activities sustain the respiratory metabolism needed for rhizosphere competence [64]. Furthermore, strain PP10 ability to utilize diverse carbon sources such as glucose, fructose, sucrose, maltose, and mannitol highlights a metabolic versatility that enhances its ecological fitness [61].

Dual culture assay revealed that *C. oceanisediminis* PP10 inhibited *Fusarium* mycelial growth by 60% through putative antifungal metabolites and hydrolytic enzymes. Although antagonism is documented in other *Cytobacillus* strains [21], this is the first report detailing the specific biocontrol efficacy of *C. oceanisediminis* PP10 against *Fusarium* sp.

Evaluated for growth-promoting traits, *C. oceanisediminis* PP10 produced significant quantities of IAA (130 µg/mL) and GA (105 µg/mL). [44] reported that phytohormone-producing rhizobacteria not only enhance plant growth but also exhibit notable activity against fungal phytopathogens. Comparable observations have been documented in PGPR-associated *Bacillus* and related *Cytobacillus* spp. [51].

Though soil phosphorus availability is often restricted by immobilization [22], rhizobacteria can mobilize bound minerals by lowering rhizospheric pH through organic acid and proton secretion [59]. Highlighting this functional trait, *C. oceanisediminis* PP10 released 150 µg/mL of soluble phosphate (solubilization index: 3.5). These results underscore its potential to enhance nutrient acquisition in *P. ovata*. Similar findings in related *Bacillus* and *Cytobacillus* species highlight phosphate solubilization as a pivotal functional trait for improving nutrient use efficiency and plant performance [23].

Microbial ammonia production enhances nitrogen availability, rhizosphere competence, and indirect antagonism against phytopathogens [50]. Consistent with these observations, strain PP10 capacity for ammonia production underscores its role in nutrient dynamics and biological disease management. This has been widely documented among related *Cytobacillus* species [39].

Furthermore, since biofilm formation is fundamental to persistent root colonization, stress resilience, and pathogen suppression [55], strain PP10-positive phenotype in both CRA and TCP assays confirms its capacity to establish stable rhizospheric associations.

Excessive soil salinity reduces global crop productivity by 20-50 % through osmotic stress, ionic imbalance, and impaired nutrient acquisition [26, 48]. While halotolerant rhizobacteria counteract these stressors to enhance host resilience [28], *C. oceanisediminis* PP10 exhibited growth up to 13% NaCl concentration [38].

Through the active secretion of hydrolytic enzymes such as protease and lipase, *C. oceanisedeminis* PP10 may exert antagonistic activity, highlighting its functional biocontrol potential [8, 30].

Root-associated *Cytobacillus* species produce diverse secondary metabolites that enhance rhizospheric competitiveness and biocontrol [34]. Among these, the lipophilic phenolic compound 2,4-di-tert-butylphenol disrupts fungal cell integrity [40, 62]. Notably, [12] demonstrated that this metabolite inhibits mycelial growth and spore germination of *Fusarium oxysporum* with an efficacy comparable to the commercial fungicide Bavistin. This antagonistic arsenal is complemented by rhamnolipids – bioactive glycolipid biosurfactant that destabilizes fungal membranes while promoting root colonization and nutrient uptake [33, 43, 49]. Notably, *C. oceanisediminis* PP10 harbors the siderophore desferrioxamine A1, which chelates  $\text{Fe}^{3+}$  with high affinity, restricting iron availability to phytopathogens and suppressing their growth [24, 35].

Volatile organic compounds are critical mediators in PGPR biocontrol, driving pathogen suppression and competitive colonization [45, 1]. To elucidate this biochemical

mechanism, GC-MS analysis was employed, revealing that *C. oceanisediminis* PP10 yields a diverse profile of volatile and semi-volatile metabolites. Among these, fatty acids, specifically palmitic acid and stearic acid, exhibit documented antimicrobial and antifungal activities [65, 27]. Additionally, detected compounds like 2-piperidinone exhibit antifungal properties, disrupting pathogen development [13, 53].

Plant inoculation assays bridge in vitro characterization and field application by assessing PGPR potential under conditions that closely mimic the natural rhizosphere. In this study, inoculation with *C. oceanisediminis* PP10 significantly enhanced seed germination, root/shoot length, vigor index, and chlorophyll content in *P. ovata* compared to controls. This growth-promoting effect is attributed to PP10's multifaceted PGPR traits, including IAA, phosphate solubilization, ammonia production, biofilm formation, and bioactive metabolite synthesis, which collectively improve nutrient availability and root development [41]. While plant growth promotion is well documented for *Bacillus* and *Cytobacillus* sp. This is the first report on the symbiotic interaction between *Cytobacillus oceanisediminis* and *Plantago ovata*, representing a novel contribution to sustainable isabgol cultivation.

## **5 Conclusion**

This study identifies *Cytobacillus oceanisediminis* PP10 as a multifunctional, halotolerant PGPR associated with the rhizosphere of *Plantago ovata*. Its ability to tolerate high salinity, exhibit antagonistic activity against fusarium sp., express multiple PGP traits, and produce diverse bioactive metabolites highlights its potential role in promoting plant health under biotic and abiotic stress conditions. Importantly, PP10 bacterization significantly improved the growth and biomass of *P. ovata* under pot conditions. These findings expand the current understanding of the plant beneficial potential of *C. oceanisediminis* and provide a basis for its further evaluation as a microbial inoculant for sustainable cultivation of *P. ovata* and other crops. To the best of our knowledge, this study represents the first report of *C. oceanisediminis* isolated from the rhizosphere of *P. ovata* and exhibiting combined plant growth promoting, halotolerant and antifungal properties.

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### **Contributions**

P.P. contributed to conceptualization, investigation, formal analysis, and writing - original draft; A.P. contributed to writing - review and editing; C.K.J. contributed to writing – review and editing and supervision. All authors have read and approved the final version of the manuscript.

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### **Data availability**

Datasets generated in this study will be available on request to corresponding author.

### **Ethics declarations**

### **Ethics approval and consent to participate**

Sampling was strictly limited to rhizosphere soil of commercially cultivated *Plantago ovata* from private farmland in Surendranagar district, Gujarat, India, with landowner consent; no plant tissues or wild specimens were harvested, rendering herbarium deposition and voucher specimens not applicable. The host plant was authenticated by the faculty of the

Department of Botany, Gujarat Arts and Science College, Ahmedabad, adhering to institutional and national guidelines.

### **Competing interests**

The authors declare no competing interests.

### **Consent for publication**

Not applicable.

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