

Exploring the Potential of *Bacillus velezensis*, an Endophytic Bacteria Isolated from *Alternanthera philoxeroides* for Plant Growth Promotion and Bioremediation Properties

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Abstract

The current work was done to analyse the bioremediation and plant growth promotion (PGP) traits of endophytic bacteria isolated from *Alternanthera philoxeroides* from Bellandur Lake, Bangalore, India. Twenty-nine endophytic bacteria were isolated and tested for their PGP traits like indole acetic acid (IAA), ammonia, nitrogen fixation, 1-aminocyclopropane-1- carboxylate (ACC) deaminase production, phosphate solubilization etc. Endophytic bacterium, BEBAphL1 obtained from leaves of *A. philoxeroides* exhibited significant plant growth promotion properties and the isolate was identified as *Bacillus velezensis* OQ874364 using 16S rRNA sequencing. The bacterium showed potential IAA, ammonia production, nitrogen fixation, phosphate solubilization, and ACC deaminase production. The results indicate that this endophyte is promising as a growth-promoting inoculant, reducing the reliance on chemical inputs in conventional agricultural practices while enhancing nutrient uptake and stress resilience in plants. *B. velezensis* exhibited tolerance to high levels of chromium (500 mg/L) and NaCl (15%) and was also able to decolourize Congo red by 70% at 0.005% concentration of dye. Characterization of dye samples pre- and post-bacterial treatment was done using Fourier-transform infrared spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The findings of the study indicate that *B. velezensis* shows promise as a plant growth stimulator capable of withstanding heavy metal exposure and breaking down dyes.

Keywords: Endophyte, Bellandur Lake, Congo Red, Chromium Tolerance, IAA production

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INTRODUCTION

Endophytes are microorganisms that reside inside plants, where they establish a symbiotic relationship with their hosts. They do not induce pathogenicity in host plants, and in return, plants provide nutrients to help them grow and survive.¹ Endophytic bacteria can reduce the toxicity and evapotranspiration of pollutants by breaking them down. Endophytic bacteria have been found to exhibit good efficacy in breaking down pollutants within plants.^{2,3} In addition, they show plant growth-enhancing properties like production of IAA, siderophore, ammonia, nitrogen fixation, ACC deaminase, potassium solubilization, phosphate solubilization, etc. which further benefits bacterial-assisted plant growth promotion.^{4,5}

The industrial revolution has led to significant advancements in meeting the needs of the growing population. The rapid growth of textile industries and the widespread use of artificial dyes have caused a concerning issue: the release of wastewater containing these dyes, leading to pollution. This pollution, arising from the discharge of dye-laden wastewater, has become a significant concern.⁶ Biological, physical and chemical techniques were used to eliminate dyes. Researchers have shown a great interest in biological methods for removing dyes, which are sustainable and economically viable. These methods encompass the utilization of microbial biomass for microbial cultivation, the adsorption of dyes by either living or non-living biomass, and enzymatic degradation. Fungi and bacteria are the most commonly studied microbes for dye removal because of their widespread occurrence and ability to adapt to different environments.^{7,8} Microorganisms and their enzymes have the ability to eliminate significant constituents like phenolics and aromatic amines found in dyes.⁹ Different types of microorganisms, including *Bacillus*, *Enterobacter*, *Candida* and *Aspergillus* etc. have been identified as capable of biologically removing colour from textile dyes. *Bacillus* sp., in particular, is well-known for its capacity to effectively decolorize dyes, especially those of the azo variety.¹⁰ The azoreductase enzyme derived from *Bacillus velezensis* has exhibited its ability to decolorize various azo dyes with different chemical

structures, including Congo red, methyl orange, methyl red, and crystal violet. This enzyme shows promising potential in efficiently removing colour from a wide range of azo dyes.¹¹ Bacteria isolated from locations contaminated with dyes have been found to be valuable resources for degradation, as indicated by various studies. Studies focused on phytoremediation have explored its application in treating wastewater from the textile industry. These studies have highlighted the significance of utilizing plants harbouring the highest number of endophytes, as they have the potential to enhance the level of degradation achieved.¹² Naturally occurring heavy metals like zinc, copper, molybdenum, manganese, cobalt, and nickel play crucial roles in various biological processes. However, excessive levels of these elements, when combined with other harmful metals such as arsenic, lead, chromium, cadmium etc., can negatively impact plants and animals.¹³ Endophytic bacteria are studied their potential role in enhancing heavy metal tolerance in crops. By harnessing the beneficial properties of endophytic bacteria, it is possible to develop sustainable strategies for improving crop performance under heavy metal stress.¹⁴ To our current understanding, our research stands as a pioneering achievement in the isolation of endophytic *Bacillus velezensis* strains that possess remarkable traits such as promoting plant growth, tolerating high salt concentrations, exhibiting resistance to heavy metals, and displaying the ability to decolorize dyes.

Bellandur Lake, which is the biggest man-made water body in Bangalore City spanning over an area of 367 hectares, has been adversely affected by the discharge of untreated or poorly treated domestic and industrial wastewater from its surrounding areas. As a consequence, the water quality of this Lake has been severely impacted, posing a significant threat to the ecosystem and the human population residing in the vicinity.¹⁵ *Alternanthera philoxeroides* is one of the abundant plant species found in Bellandur Lake and recognised as invasive in the European Union Concern (EU Regulation 1143/2014).¹⁶ Currently, there is limited understanding regarding endophytic bacterial isolates present in *A. philoxeroides* plants. It is uncertain whether the endophytic bacteria found in *A. philoxeroides*,

can promote plant growth as well as be utilized to facilitate the heavy metal absorbance and degradation of dyes. Further research is essential to explore this topic. Our current objective was to isolate endophytic bacteria from *A. philoxeroides* growing in contaminated Bellandur Lake and to determine their bioremediation and PGP properties.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium hypochlorite, Tween 80, tryptophan, nutrient broth, ammonium sulphate, Congo red, gelatin, IAA and Chromium sulphate were bought from HIMEDIA (India). All reagents like Salkowski reagent, Kovac's reagent, Nessler's reagent and HPLC grade solvents like ethyl acetate were purchased from HIMEDIA (India). Other chemicals and reagents used in this study were also bought from HIMEDIA (India). All reagents and chemicals used in this study were of analytical grade. For spectrometric readings, Shimadzu UV-1800UV/Visible Scanning Spectrophotometer (India) was used.

Isolation of Endophytic Bacteria

Aquatic plants were obtained from Bellandur Lake, Bangalore, India (Lat 12° 56'24.4824" Long 77° 40'30.9144"), which is one of the largest and most polluted lakes in the region. The most abundant plant species found in the lake, *Alternanthera philoxeroides* (Mart.) Griseb, was

selected for the isolation of endophytes (Figure 1). The plant parts were washed with running tap water, treated with Tween 80 for five minutes, and rinsed again using sterile distilled water. Surface sterilization of the plant parts was done inside a Laminar Air Flow (LAF) chamber. The plant samples were then sliced into small 1 to 2 mm pieces and immersed in ethanol (70%) for 30 seconds.¹⁷ Following several rinses with sterile water, the plant samples underwent a one-minute treatment with sodium hypochlorite containing 1% chlorine. After another round of washing with sterile water, the final rinse water, measuring 100 µl, was spread evenly onto a nutrient agar (beef extract 3 g, yeast extract 3 g, peptone 5 g, NaCl 5 g, agar 15 g, and dH₂O 1 L with pH 7.0) as control.¹⁷ The sterilized plant parts were blot dried and inoculated onto nutrient agar, LB agar, and King's B agar plates. The plates were incubated (37°C) for a week and examined for the growth of bacterial colonies. Unique colonies were chosen and purified for additional research.

Biochemical and Molecular Characterization of Endophytic Bacteria

The isolates were subjected to morphological characterization, and different biochemical tests, like Indole, Methyl Red, Citrate and Voges Proskauer tests, were conducted, as well as Gram staining and motility experiments. The endophytic bacterium that exhibited the most promising plant growth promotion and bioremediation properties was selected for

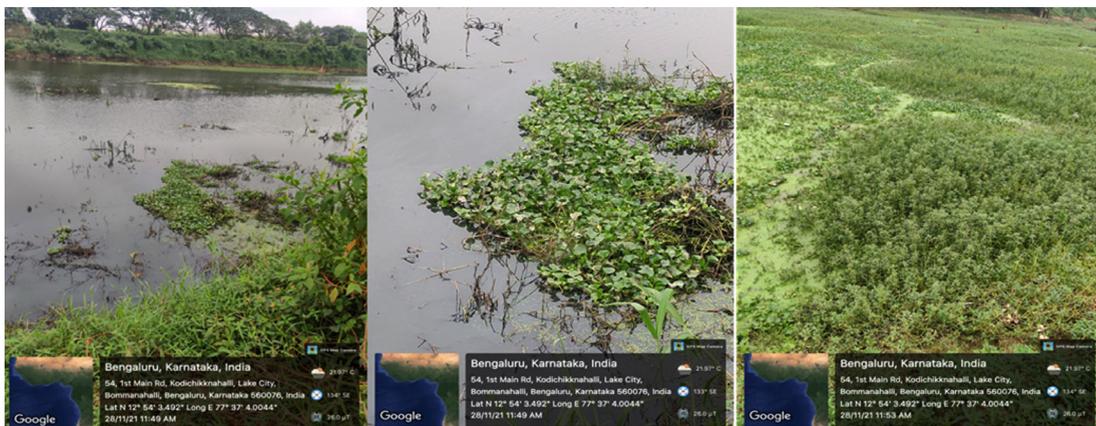


Figure 1. Sampling site for endophytic bacterial isolation from Bellandur Lake, Bangalore, India (Lat 12°56'24.4824" Long 77° 40'30.9144")

molecular identification. In order to confirm the purity of the isolated DNA, agarose gel (1%) was used to evaluate it. The amplification of the 16S rRNA gene fragment was accomplished by utilizing 16S rRNA-F and R primers. The forward and reverse primers were utilized for sequencing reaction using the BDT v3.1 cycle sequencing kit on the ABI 3720xl Genetic Analyzer. The resulting consensus sequence was analysed through the NCBI GenBank database using BLAST analysis, based on the maximal identity score, and the initial ten sequences were aligned using ClustalW (<http://www.clustal.org/clustal2>), a software program for multiple sequence alignment. Distance matrix and phylogenetic tree were created using MEGA 10 (www.megasoftware.net). The accession number was obtained by submitting the sequence information to the NCBI database.^{18,19}

Estimation of Ammonia and Indole Acetic Acid (IAA) Production

The isolated endophytic bacteria were analysed for the production of IAA on a qualitative and quantitative basis. The cultures were inoculated into nutrient broth enriched with tryptophan (0.5% w/v) and incubated at 37°C for

48 h. The cultures were centrifuged at 8000 rpm for 15 min. Salkowski reagent (0.5 M FeCl₃:dH₂O: H₂SO₄ in 1:50:30 ratio) (2 mL) was added to 1 mL supernatant. Positive isolates showed red colour formation and the quantity of IAA was assessed by measuring and estimating the absorbance of the sample at 530 nm by using a Shimadzu UV-Visible Spectrophotometer.^{20,21}

Ammonia production by endophytes was determined using Nessler's reagent test. The samples were grown in nutrient broth at a temperature of 37°C and 120 rpm. The cultures then underwent centrifugation at 10,000 rpm for 10 minutes. The supernatant was taken and treated with 0.5 mL Nessler's reagent, followed by observation for the development of a yellowish-brown colour. Quantitative determination of ammonia production was estimated using a Shimadzu UV-Visible spectrophotometer at 430 nm.²²

Estimation of Nitrogen Fixation, Phosphate and Potassium Solubilization by Endophytic Bacteria

The potential of isolates to solubilize phosphate was checked based on a modified method²³. The endophytic isolates were inoculated

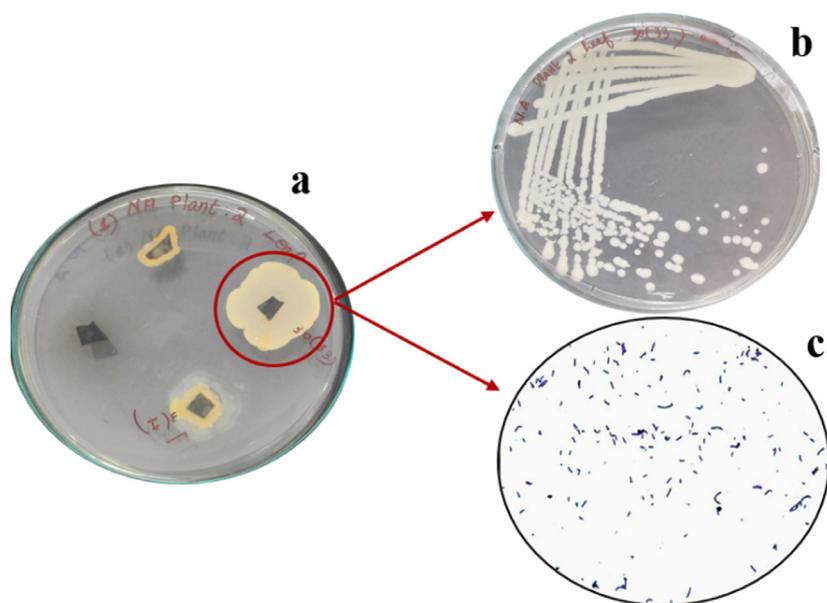


Figure 2. Isolation of bacterial endophyte from *Alternanthera philoxeroides* (a) Bacterial growth from leaves of *Alternanthera philoxeroides* in nutrient agar media (b) pure colony isolation using quadrant streak method (c) Gram stain test of the isolated bacterial endophyte

to Pikovskaya medium (yeast extract 0.5 g, $\text{Ca}_3(\text{PO}_4)_2$ 5 g, dextrose 10 g, $(\text{NH}_4)_2\text{SO}_4$ 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g, KCl 0.2 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.00001 g, MnSO_4 0.0001 g, agar 15 g, dH_2O 1 L; pH 7.0 containing 1mL 0.5% bromophenol blue). Incubation was done at 37°C for two days, and then checked for the formation of a yellow halo zone surrounding the colonies. This indicated that the phosphate had been solubilized. The Edi-Premono formula²⁴ was used to calculate the solubilization index.

Phosphate Solubilization Index (SI) = (Colony diameter + Halo zone diameter) / Colony diameter

Bacterial endophytes were inoculated onto Jensen’s medium to check nitrogen fixing ability.²⁵ Isolates were incubated for seven d at 37 °C and observed for bacterial growth which was interpreted as a positive result. Solubilization of potassium by endophytic bacteria were tested *in vitro* using Aleksandrov media.²⁶ Endophytes were inoculated to Aleksandrov media (glucose 5 g, FeCl_3 0.1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.005 g, CaCO_3 2 g, $\text{Ca}_3(\text{PO}_4)_2$ 2 g, mica powder 3 g, agar 20 g, dH_2O 1 L, pH 7.2) and incubated for three to five days at 37 °C. The clearzone around the colonies was confirmed as positive for potassium solubilization.

Estimation of ACC Deaminase and Siderophore Production

ACC deaminase production by bacterial endophytes were screened *in vitro* using DF

minimal salt media (KH_2PO_4 4 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g, Na_2HPO_4 6 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, MnSO_4 10 µg, H_3BO_3 10 µg, CuSO_4 50 µg, ZnSO_4 70 µg, gluconic acid 2 g, MoO_3 10 µg, Glucose 2 g, citric acid 2 g, agar 12 g, dH_2O 1 L).²⁷ The media was supplemented with ammonium sulphate (2 g/L).²⁸ The isolates were incubated at 37°C for 3 d, and the growth of bacteria were considered as a positive result.

Siderophore production by isolates was analysed using a modified method.²⁹ The isolates were inoculated into nutrient broth and subjected to incubation at 37°C for 24 h, after which the culture underwent centrifugation at 10,000 rpm for 10 min. After adding 2 mL of 2% (w/v) FeCl_3 to 5 mL of the supernatant, the solution was observed for the appearance of a red or purple colour, which was considered indicative of a positive result.

Screening of Extracellular Enzyme Production by Endophytic Bacteria

Protease production by endophytic isolates was screened using skim milk agar media (skim milk powder 28 g, tryptone 5 g, yeast extract 2.5 g, glucose 1 g, agar 18 g, dH_2O 1 L, pH 7.2). Bacteria was inoculated to this media and incubated at 37°C for 48 h. The halozone around the colonies was considered as a positive result.³⁰ Tributyrin media (yeast extract 3 g, peptone 5 g, tributyrin oil 4 mL, agar 18 g, dH_2O 1 L, pH 6.8) was used to screen lipase producing bacterial

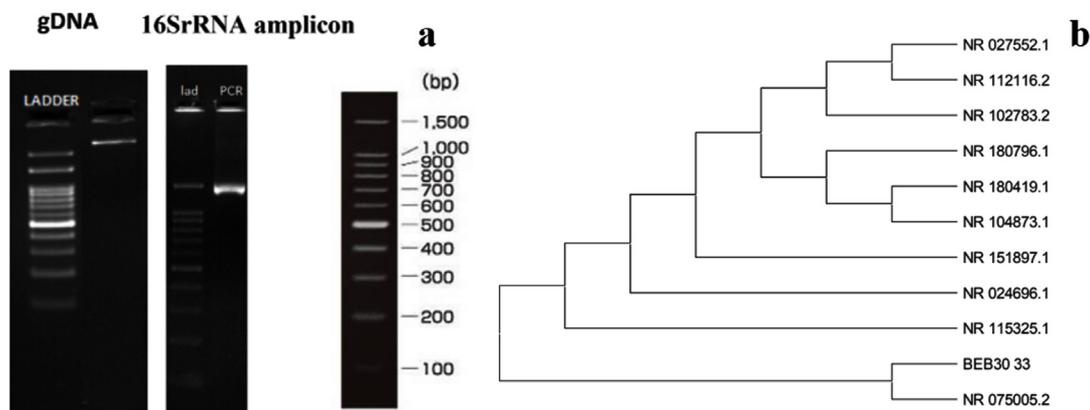


Figure 3. (a) Species identification of isolated endophytic bacteria BEBAphL1 isolated from leaves of *Alternanthera philoxeroides*: Genomic DNA isolation and comparison with DNA ladder (b) Phylogenetic tree of the isolated endophyte bacteria

isolates. Following incubation of the plates at 37°C for 48 h, a positive result was determined by the observation of a halo zone surrounding the colony.³⁰

A modified medium was employed to assess the cellulose production capability of endophytic bacteria.³¹ The isolates were inoculated onto cellulose Congo red media, which consists of cellulose 2 g, MgSO₄·7H₂O 0.25 g, KH₂PO₄ 0.5 g, Congo red 0.2 g, gelatin 2 g, agar 15 g, dH₂O 1 L, pH 6.8. Subsequently, the media was incubated at 37 °C for 48 h, and identifying a clear zone encircling the colonies confirmed a positive result. Starch agar media (peptone 20 g, starch 20 g, agar 20 g, dH₂O 1L, pH 6.8) was used to analyse the production of amylase by endophytes. The media was incubated at 37°C for 48 h, and after bacterial growth, 1% (v/v) Gram's iodine was added to flood the colonies. A positive outcome was inferred from the existence of a distinct halo zone surrounding the colonies.³²

Enzymatic index (EI) was calculated for these isolates.³³

$$EI = (\text{Halo zone diameter} / \text{Colony diameter (cm)})$$

Halotolerance Properties of Endophytic Isolate

To assess the capacity of bacterial isolates to endure elevated salt levels, spot inoculation was conducted on nutrient agar plates supplemented with NaCl (5%, 7%, 10%, 12%, 15%, and 20% (w/v)). The media plates were incubated at 37°C for 5 d, and the growth of bacteria was observed every 24 h.³⁴

Heavy Metal Tolerance of Endophytic Bacteria

Bioremediation properties of the best PGP bacterium were determined. The tolerance of the bacterium to chromium was evaluated using the agar-dilution method. Nutrient agar medium was supplemented with varying concentrations of chromium, spanning from 100 to 3000 mg/L. After autoclaving and cooling the media metal salts (Cr₂(SO₄)₃) were added. The media was inoculated and cultured at 37°C for 48 h. Observation of bacterial growth was conducted to identify the metal concentration that completely halted the growth, which was subsequently designated as the Minimum Inhibitory Concentration (MIC).³⁵

Estimation of Dye Degradation Potential of Endophytic Bacterium

The initial screening of the endophytic isolate's dye degradation potential was conducted by spot-inoculating the bacterium on MSM agar plates containing different azo dyes. MSM media was composed of KH₂PO₄ 4 g, K₂HPO₄ 5 g, CaCl₂·2H₂O 0.5 g, MgSO₄·7H₂O 0.2 g, NH₄Cl 0.4 g, dH₂O 1 L, pH 7.2. The degradation potential of the isolated bacterium was evaluated against three azo dyes, namely Congo red (CR), crystal violet (CV), and reactive red (RR), at different concentrations (0.005, 0.01, 0.05 g /100 mL). The inoculated media was incubated at 37°C for 48 h and observed for the presence of a decolorization zone.^{36,37}

Decolorization of CR Dye with Isolated Bacterium

To determine the extent of decolorization of CR by the isolated bacterium, a 1% overnight

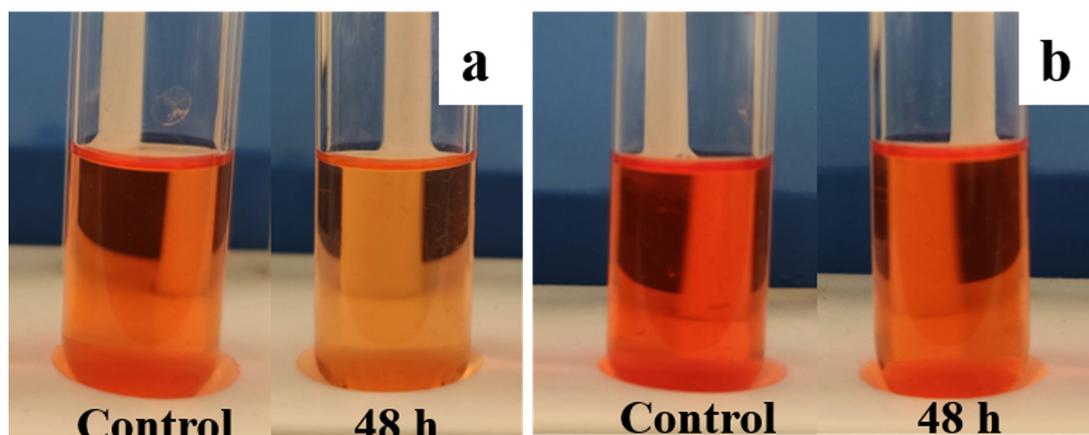


Figure 4. Comparison of control and 48 h bacterial (*Bacillus velezensis*) incubated dye samples. (a) results obtained for dye samples with a concentration of 0.005% (b) results obtained for dye samples with a concentration of 0.01%

culture was inoculated into a medium composed of yeast extract 10 g and NaCl 5 g, along with different concentrations of the dye (0.005%, 0.01%, and 0.05%). Subsequently, the medium that had been inoculated was kept at incubator at 37°C and 120 rpm 24 to 48 h. Following incubation, the bacterial culture was centrifuged at 8000 rpm for 10 minutes, and the resultant supernatant was taken. The decolorization of the dye in the supernatant was assessed by measuring its absorbance at 497 nm using a Shimadzu UV-visible spectrophotometer. The percentage of dye decolorization was detected using the following equation.

$$\text{Decolourization (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

where R_1 is the initial absorbance, R_2 is the final absorbance of CR dye decolorization.³⁸

Characterization of treated and untreated dye samples: GC-MS and FTIR Analysis

Samples of untreated and bacterial-treated CR dye were obtained and centrifuged at 8000 rpm for 10 min sat 4°C.³⁹ Treated samples are samples that consist of 0.005% Congo red and 1% bacterial culture which are then incubated for

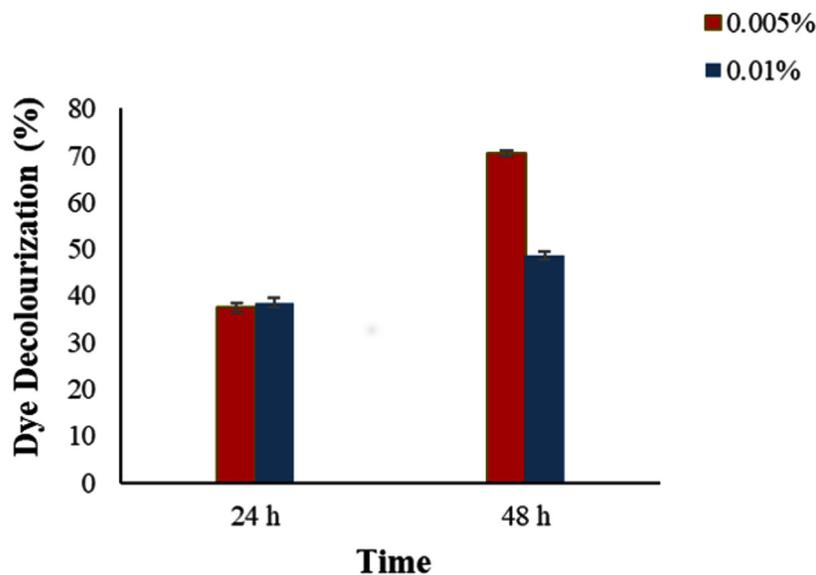


Figure 5. Decolourization of congo red by bacterial endophyte *Bacillus velezensis* isolated from *Alternanthera philoxeroides* at specific time intervals and different dye concentrations

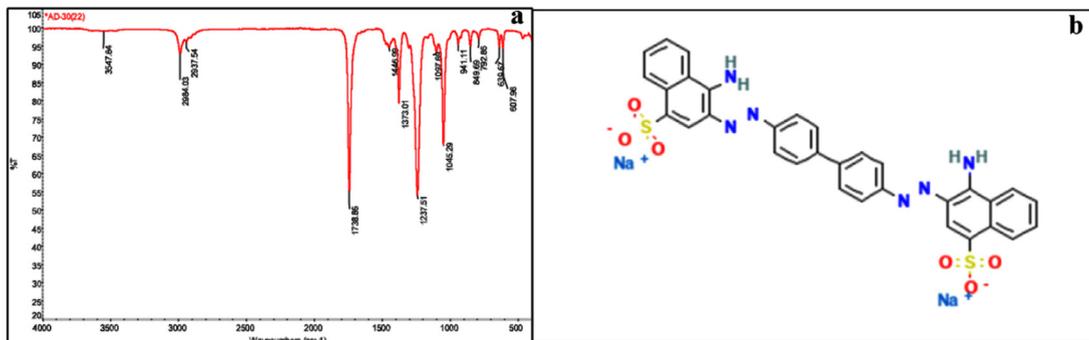


Figure 6. (a) FTIR spectrum of dye sample treated with endophytic *Bacillus velezensis* isolated from leaves of *Alternanthera philoxeroides* (b) Chemical structure of Congo red dye selected for decolourization study using *Bacillus velezensis*

48 h. Untreated samples do not contain bacterial culture. The obtained supernatant was utilized to extract the compounds from the samples using a liquid-liquid extraction technique, involving the use of an equal quantity of ethyl acetate and the sample.³⁹ The extract was evaporated to dryness and used for FT-IR whereas 5 μ L extract was taken for GC-MS analysis.

GC-MS Analysis

The analysis conducted using the Clarus 680 GC involved a fused silica column packed with Elite-5MS, which is a mixture of 5% biphenyl and 95% dimethylpolysiloxane, and had dimensions of 30 m x 0.25 mm ID x 250 μ m df. A consistent flow rate of 1 mL/min of helium gas was employed as the carrier gas. Throughout the chromatographic process, the temperature of the injector was adjusted to 260°C, and a 1 μ L sample extracted was injected into the instrument. The temperature settings for the oven were programmed in the following manner: an initial temperature of 60°C was maintained for 2 min, after which it was increased at a rate of 10°C per minute until reaching 300°C. This temperature was then maintained for 6 min. The mass detector was utilized with specific temperature settings, including a transfer line and ion source temperatures of 240°C each. The electron impact ionization mode was employed with an energy of 70 eV. During the analysis, the scan time for each measurement was set to 0.2 seconds, with a scan interval of 0.1 sec. The mass fragments were recorded within the range of 40 to 600 Da. The obtained spectra were compared with the database of compounds saved in the GC-MS NIST (2008) library.

Fourier-transform infrared spectroscopy (FTIR) Analysis

The structural and functional groups in the degraded dye sample were analysed using FTIR-ATR spectra (ThermoScientific Nicolet i550 spectrometer). The spectral data was captured within a range of wavelength of 400 to 4000 cm^{-1} with a resolution of 8 cm^{-1} .

Statistical Analysis

All tests were performed in triplicates, and the average value and standard deviation were calculated using MS Excel for data analysis.

RESULTS AND DISCUSSION

Isolation of Endophytic Bacteria

In our study, we isolated bacterial endophytes from different plant parts of *Alternanthera philoxeroides* (Mart.) Griseb after surface sterilization. We used three media, including nutrient agar, LB agar, and King's B agar for isolation. We have successfully obtained 29 bacterial endophytes with unique morphological features from leaves, shoots, and roots of *A. philoxeroides*. BEBAphL1 exhibited noteworthy abilities in promoting plant growth and bioremediation. Thus, we have discussed the test outcomes of this bacterial endophyte. BEBAphL1 was isolated from leaves of *A. philoxeroides*. The sampling site is shown in Figure 1. In a study, endophytic bacteria were isolated from specimens of *Alternanthera philoxeroides* that were grown in soil containing hydrocarbons. The bacteria were found to thrive in those conditions and could potentially be used for bioremediation.⁴⁰ In another study, fungal endophytes were isolated from the same plant species that produced laccase enzymes.⁴¹

Biochemical and Molecular Characterization of Endophytic Isolate

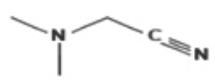
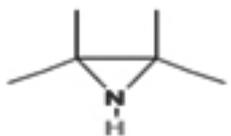
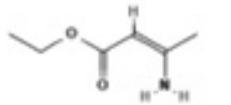
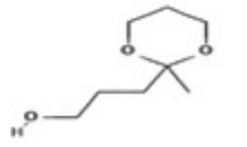
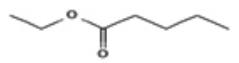
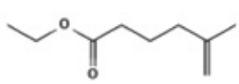
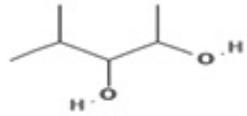
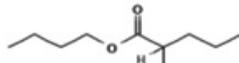
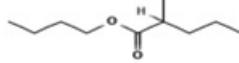
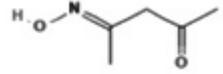
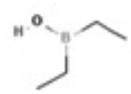
Colony morphology and biochemical test results of the endophytic isolate were checked (Figure 2). The isolated bacterial colony was white in colour, medium sized and had mucoid consistency. The colony was circular with an entire margin and had umbonate elevation. The isolate was Gram-positive, rod-shaped and motile. Negative results were shown for indole and citrate tests. Voges Proskauer and Methyl red tests were negative.

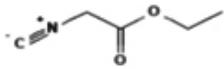
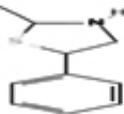
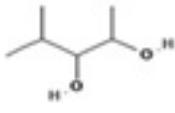
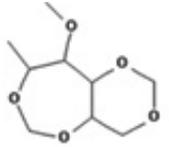
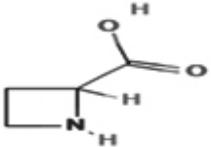
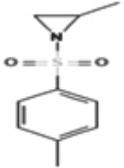
The potential plant growth promoter with bioremediation properties has been identified through 16S rRNA sequencing, and the sequence data was deposited in Genbank (Figure 3). Based on BLAST analysis, BEBAphL1 showed a 99.64% sequence similarity to *Bacillus velezensis* strain FZB42 (NR_075005.2). The Genbank accession number for the sequenced isolate is OQ874364.

IAA and Ammonia Production

The production of IAA is a significant characteristic of rhizospheric and endophytic

Table. Compounds present in untreated and treated dye samples analysed using GC-MS

Compounds	Retention Time	Untreated Dye Sample	Treated Dye Sample	2D Structure
Dimethyl aminoacetonitrile	1.309	+	-	
Aziridine	1.309	+	-	
Ethyl 3-aminocrotonate	1.309	+	-	
1,3-Dioxane-2-propanol, 2-methyl-	1.309	+	-	
Ethyl pentanoate	1.664	+	-	
Ethyl isovalerate	1.664	+	-	
Ethyl 5-methyl-5-hexenoate	1.664	+	-	
4-methylpentane-2,3-diol	1.904	+	-	
Butyl 2-methylvalerate	1.904	+	-	
3-Methoxypropanoic acid	1.904	+	-	
Acetylacetone	1.904	+	-	
Diethylborinic acid	28.639	+	-	

Ethyl isocyanoacetate	28.639	+	+	
2,2- Dimethylundecane	28.639	+	-	
2-Methyl-5-phenyl-thiazolidine	28.639	+	-	
1,1-Dimethylsiletane	1.909	-	+	
4-methylpentane-2,3-diol	1.909	-	+	
Methoxycarbonyl isothiocyanate	1.909	-	+	
1,3: 2,5-Dimethylene-4-methyl-d-rhamnitol	1.909	-	+	
Allylamine	28.574	-	+	
Cyclopentylamine	28.574	-	+	
Azetidine-2-carboxylic acid	28.574	-	+	
2-Methyl-1-Tosyl-Aziridine	28.574	-	+	

Where, - is absent; + is present

bacteria, which play a crucial role in promoting and facilitating plant growth.⁴² IAA production of endophytic bacterium *B. velezensis* was 30.42 ± 2.09 $\mu\text{g}/\text{mL}$ in tryptophan-containing media. The plant growth promotion properties of endophytic *Bacillus velezensis* Lle-9, which was obtained from *Lilium leucanthum*, were assessed, and the bacterium produced IAA. Additionally, tryptophan present in the medium resulted in an increased rate of IAA production.⁴³ Endophytic *Bacillus velezensis* D4 isolated from branches of apple and pear showed plant growth promotion properties but IAA production was not present in that particular strain.⁴⁴ Endophyte *B. velezensis* was screened for its ability to produce ammonia, an essential metabolite that assists in meeting the nitrogen requirements of plants. This was done by adding Nessler's reagent to peptone water to detect ammonia production activity.⁴⁵ Quantitative estimation of ammonia produced was estimated based on the ammonium sulphate standard. It was found to be 0.89 ± 0.08 μmol . A study was conducted to determine the potential of *B. velezensis* BACO3 to enhance plant growth and it was discovered to have the ability to generate ammonia. The production of ammonia is connected to the accumulation of nitrogen, elongation of plant roots, and increased biomass production.⁴⁶

Nitrogen Fixation, Phosphate and Potassium Solubilization by Endophyte

Nitrogen is a vital component of nucleic acids, enzymes, proteins, and chlorophyll that enables photosynthesis in plants. Although nitrogen is present in large quantities in the Earth's atmosphere it exists mainly in the inert form of dinitrogen (N_2), which cannot be utilized by plants. Nitrogen fixation is the process by which Nitrogen gas is transformed into these usable forms, making it a crucial process for the survival of life on Earth. Few endophytic bacteria possess nitrogen-fixing abilities, which potentially promote the growth of plants. The nitrogen fixation ability of *B. velezensis* was assessed using Jensen's media. The bacterium exhibited growth in this media, indicating a positive result for nitrogen fixation. *B. velezensis* strain OEE1, which was obtained from olive trees, was evaluated for its nitrogen fixation capacity in Jensen's media that lacked nitrogen, and was

observed to yield a positive result.⁴⁷ *Bacillus velezensis* SR18 isolated from rhizospheric soils of Indo Gangetic plains (IGPs) showed a phosphate solubilization index of 8.5.⁴⁸

The process of inorganic phosphate solubilization by bacteria and fungi associated with plants is a vital aspect of plant growth promotion.⁴⁹ Our research involved examining the ability of endophytic *B. velezensis* to solubilize phosphate. The results showed that it had a phosphate solubilization index of 1.75 ± 0.05 . The plant growth promotion properties of *Bacillus velezensis* GL5, isolated from *Gnetum gnemon* L, was evaluated including its ability to solubilize phosphate. However, it showed negative results for phosphate solubilization.⁵⁰

Potassium fertilizers make up 18% of the total consumption of fertilizers which contain nitrogen, phosphorus, and potassium (N, P, and K) worldwide. Although the soil contains substantial amounts of potassium (K), only a small portion of it is actually accessible for plants to absorb.⁵¹ It was found that the *B. velezensis* strain isolated in our study did not possess the capacity to solubilize potassium after testing it with Alexandrov's media. Earlier research has also tried to depict the potassium solubilization capability of the endophytic strain *Bacillus velezensis* ERBS51. However, the outcome of the experiment showed that the strain could not solubilize potassium which was similar to our results.⁵²

ACC Deaminase and Siderophore Production

ACC deaminase increases plant growth by decreasing levels of ethylene in plants. The enzyme converts ACC, a precursor involved in the synthesis of plant ethylene, into ammonia and α -ketobutyrate.⁵³ Reduction in ethylene and ACC promotes root growth and development. Although recent research have suggested the possibility of endophytic bacteria to produce ACC deaminase, an area of study which is still being investigated and requires further exploration.⁵⁴ The production or acquisition of siderophores is a characteristic that enables microorganisms to be effective competitors in various environments. This process involves the creation of small molecules with a high affinity for Fe (III) in conditions of iron deficiency, followed by the capture of iron-charged siderophores by the cell.^{55,56} Our research involved

assessing the ability of endophytic *B. velezensis* to produce ACC deaminase and siderophores. Upon incubation, the isolate exhibited a positive outcome for ACC deaminase production, but a negative result for siderophore production. *Bacillus velezensis* Lle-9 isolated from *Lilium leucanthum* displayed PGP properties, such as siderophore production and ACC deaminase.⁵⁷

Protease, Lipase, Cellulase and Amylase Production

Endophytic bacteria secrete extracellular enzymes, hydrolases, that help plants develop systemic resistance to prevent pathogen invasion. These enzymes have numerous applications in the food industry, pharmaceuticals, biofuels, biopesticides, detergents, and other industrial products. Over time, the methods for producing these metabolites and enzymes have been enhanced since their initial discovery.^{58,59} The results of our study indicate that *B. velezensis* exhibited lipolytic and cellulolytic characteristics, as evidenced by an enzymatic index of 2.22 ± 0.16 and 2.51 ± 0.10 , respectively. In a study, endophytic *Bacillus velezensis* HNH7 and *Bacillus altitudinis* HNH9 were isolated which showed plant growth promotion properties as well as enzyme production. The endophyte exhibited proteolytic, amylolytic, lipolytic and cellulolytic activity.⁶⁰

Halotolerance Properties of Endophytic Isolates

Soil salinity is recognized as a major abiotic stressor that can negatively affect agricultural output, microbial populations, and the economic viability of farming in impacted areas. This has become a significant issue in the agricultural sector worldwide, particularly over the past few decades. Nevertheless, there exists a possible remedy through salt-tolerant bacteria that exhibit various beneficial traits for plant growth. These bacteria can enhance the tolerance of plants to salinity stress and facilitate their growth in salinity-affected environments.⁶¹⁻⁶³ In our study, it was observed that the endophytic isolate *B. velezensis* demonstrated the ability to withstand a high concentration of NaCl up to 15%. In a study, *Bacillus velezensis* FMH2 was found to be an efficient strain in safeguarding plants against fungal pathogens and enhancing the growth of

tomato seedlings, in stressful environments with high levels of salt.⁶⁴

Heavy Metal Tolerance of Endophytic Bacterium

One of the main concerns of industrialization is the build-up of heavy metals, particularly chromium in soils and lakes. The toxicity of heavy metals in plants can result in various detrimental effects such as inhibition of growth, chlorosis, necrosis, disruption in the balance of mineral nutrients leading to impaired enzymatic activities etc. Endophytes with the ability to tolerate or degrade heavy metals to protect the host plants are an area that has received limited research.⁶⁵ Our study found that *B. velezensis* demonstrated the ability to withstand metal salt $\text{Cr}_2(\text{SO}_4)_3$ at concentrations as high as 500 mg/L. *Bacillus velezensis* TW17, which was obtained from samples of sludge from a tannery, showed the ability to degrade chromium, with the highest rate of degradation occurring on the seventh day of observation.⁶⁶ Many endophytic bacteria are also halotolerant and has high potential to be used as green inoculants.⁶⁷

Dye Degradation

To assess the ability of *B. velezensis* in dye degradation, crystal violet, reactive red, and Congo red were used in the initial screening. The results revealed that the bacterium only exhibited a zone of clearance when grown in media containing Congo red, indicating its ability to degrade Congo red specifically (Figure 4). *B. velezensis* was found to have the ability to degrade Congo red dye, so we studied further. The amount of dye broken down by the bacterium at 24-h and 48-h intervals was measured and recorded the results as percentages of decolourization. Specifically, the decolourization percentages for dye concentrations of 0.005% and 0.01%, were done and the results are provided in figure 5. A study identified *Bacillus velezensis* as having the ability to remove azo dyes, and found out that this ability was linked to the presence of a gene called azoR2.¹¹

FTIR

FTIR analysis described the functional groups present in bacterial-treated Congo red dye.

Ethyl acetate extract of treated Congo red dye was analysed and several characteristic peaks were obtained near 849.69 cm^{-1} , 941.11 cm^{-1} , 1045.29 cm^{-1} , 1097.69 cm^{-1} , 1237.51 cm^{-1} , 1373.01 cm^{-1} , 1446.00 cm^{-1} , 1738.86 cm^{-1} , 2937.54 cm^{-1} , 2984.03 cm^{-1} , 3547.84 cm^{-1} (Figure 6a). The peak at 1045.29 cm^{-1} corresponds to stretching vibration of S=O, while peaks at 1237 cm^{-1} , 1373 cm^{-1} , 1446 cm^{-1} represent C–N stretching, C–N bending, and N=N stretching vibrations, respectively. Additionally, the peak at 2984.03 cm^{-1} arises from the symmetric stretching of C–H bonds.⁶⁸⁻⁷⁰ The chemical structure of Congo red dye is given in Figure 6b.

GC-MS Analysis

GC-MS analysis of treated and untreated dye samples were carried out and are given in Table. Major peaks observed in untreated dye samples are dimethyl aminoacetonitrile, aziridine, ethyl 3-aminocrotonate and 1,3-dioxane-2-propanol, 2-methyl at retention time (RT) 1.309. At RT 1.664, compounds like ethyl pentanoate, ethyl isovalerate and ethyl 5-methyl-5-hexenoate. 4-methylpentane-2,3-diol, Butyl 2-methylvalerate, 3-methoxypropanoic acid and acetylacetone were found at RT of 1.904. At RT 28.639, diethylborinic acid, ethyl isocynoacetate, 2,2-dimethylundecane and 2-methyl-5-phenyl-thiazolidine were present. Compounds like hexanoate and methyl esters are utilized during the printing and dyeing process. However, dyes can have harmful effects on aquatic life, leading to a decrease in the amount of dissolved oxygen and negatively impacting both plants and animals.⁷¹ During the scouring stage of textile production, various axes, like undecane and dodecane were employed as phase changers and protective agents. These waxes serve to safeguard textile products from a range of potential threats, including water, high temperatures, nuclear radiation, biological agents and chemicals. Ethyl 3-aminocrotonate (EAC) is a crucial component in the production of numerous substances, including pharmaceuticals, dyes, and pesticides. It serves as a significant intermediate in their synthesis. 1,3-dioxane-2-propanol is a compound used as a solvent and extractor in the processing fats, waxes, and dyes. Following bacterial treatment, it was observed that the original compounds underwent mineralization while certain compounds were

converted into novel metabolic products. After degradation at RT 1.909 1,1-dimethylsiletane, 4-methylpentane-2,3-diol and methoxycarbonyl isothiocyanate and 1,3:2,5-Dimethylene-4-methyl-d-rhamnitol were present. At RT 28.574, allylamine, cyclopentylamine, azetidine-2-carboxylic acid and 2-methyl-1-tosyl aziridine were found.

CONCLUSION

Overall, the study characterized bacterial endophyte *Bacillus velezensis* OQ874364 isolated from *Alternanthera philoxeroides* grown in Bellandur Lake, Bangalore, India. The isolate possessed potential PGP properties including IAA production, ammonia production, nitrogen fixation, phosphate solubilization and ACC deaminase production. Furthermore, the isolate showed bioremediation properties like heavy metal tolerance and Congo red dye degradation along with halotolerance properties. Within 48 hB. *velezensis* successfully decolorized 70% of Congo red dye at a concentration of 0.005%, and it exhibited chromium tolerance up to 500 mg/L. These results indicate the diverse roles of endophytic *B. velezensis* in plant growth promotion, dye degradation and heavy metal tolerance. The aforementioned bacterium exhibits considerable potential for future research endeavours and can be a valuable isolate in various studies and applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SS and IP conceptualized the study. IP, SS, SB and SJ contributed to the study conception and design. IP, SB and SJ performed the experiments and wrote the manuscript. IP, SS, SB and SJ reviewed and approved the article for final publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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