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RESEARCH ARTICLE

Potential of exo-polysaccharide producing plant growth promoting bacteria in growth promotion and disease suppression of chilli

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Abstract

Bacteria producing exo-polysaccharides (EPS), isolated from the rhizosphere of chilli (*Capsicum annuum* L.), demonstrate considerable potential for enhancing plant growth and managing fungal pathogens. These bacteria exhibit a variety of plant growth-promoting traits, including the synthesis of phytohormones like indole-3-acetic acid (IAA) and gibberellic acid (GA), as well as the production of siderophores, phosphate solubilization and ammonia release. The antagonistic activity of these isolates against chilli anthracnose pathogen *Colletotrichum capsici*, was evaluated using dual plate assay. Among the isolates tested, *Bacillus halotolerans* CAJPH6 showed the maximum inhibition of *C. capsici* (72 %), followed by *Bacillus stercoris* CAJRH6 (70 %) and *Bacillus subtilis* CFORH5 (64 %). The gravimetric evaluation of EPS production showed that *Bacillus stercoris* CAJRH6 yielded 0.93 mg mL^{-1}, *Bacillus subtilis* CFORH5 produced 0.89 mg mL^{-1} and *Bacillus halotolerans* CAJPH6 generated 0.72 mg mL^{-1}. This study emphasizes the potential of EPS-producing bacterial isolates as an alternative to chemical fertilizers and fungicides, supporting sustainable and eco-friendly farming practices.

Keywords: biocontrol; chilli anthracnose; exo-polysaccharides; plant growth promotion; sustainable agriculture

Introduction

The growing interest on sustainable agricultural practices has shifted global focus towards the exploitation of beneficial microbes in enhancing plant health and productivity. Among these beneficial microorganisms, bacteria that produce bioactive extracellular polymeric substances, specifically exo-polysaccharides (EPS), are emerging as promising candidates for enhancing plant growth and providing biocontrol. These EPS-producing bacteria represent a subset of plant growth-promoting rhizobacteria (PGPR) that have garnered significant attention for their multifaceted roles in improving soil structure, enhancing nutrient uptake and conferring protection against various biotic and abiotic stresses. The unique properties of bacterial EPS, including their ability to form biofilms, retain water and chelate nutrients, make them particularly valuable in the context of sustainable agriculture, where there is a pressing need for eco-friendly alternatives to chemical fertilizers and pesticides.

The EPS are heteropolymers with an average molecular weight of 8×10^4 Da, comprising various monosaccharides such as arabinose and xylose and featuring functional groups like hydroxyl, carboxyl, N-acetyl, amine and sulfate esters (1). Many soil bacteria produce it as capsular and slime materials, which can be absorbed by clay surfaces to form a protective layer around the aggregated soil (2). EPS producing bacteria are safeguarded from water stress as they can diffuse these polysaccharides in a controlled manner to the external environment thereby improving water retention (3). EPS can form a fibrillar network that securely anchors bacteria to the root surface, facilitating their irreversible attachment and effective colonization on plant roots (4, 5). EPS helps retain excess water by promoting soil aggregation and the formation of the rhizhosheath, which acts as a protective barrier against water loss through evaporation (6). Under drought conditions, plants inoculated with EPS-producing bacteria exhibit higher levels of glucose, proline and free amino acids. Bacteria that produce EPS have become a promising resource for enhancing plant growth and providing biocontrol. EPS, a key element of bacterial biofilms, is vital at the plant-microbe-soil interface, where it helps bacteria adhere to surfaces, enhances soil aggregation and supports nutrient exchange (7). In addition, EPS enhances the survival and colonization of beneficial microbes in the rhizosphere thereby amplifying their positive effects on plant health under both normal and stressed conditions (8).

Recent studies demonstrated that EPS-producing bacteria could significantly enhance crop yields and resilience in various agricultural systems. These microorganisms have shown to improve soil aggregation, increase water retention capacity and promote the formation of beneficial microbial communities in the rhizosphere (7-9). Furthermore, the

application of EPS-producing bacteria as bioinoculants has emerged as a promising strategy for mitigating the negative impacts of climate change on agriculture, particularly in regions prone to drought and soil degradation.

Capsicum annuum L., commonly known as chilli, is a globally cultivated crop of commercial importance. However, it is highly susceptible to biotic stress caused by fungal pathogens, particularly *Colletotrichum capsici*, which is responsible for anthracnose disease and affects the yield and quality of chilli, resulting in considerable economic losses to chilli growers (10). Conventional disease management strategies relying on chemical fungicides pose numerous disadvantages including environmental contamination, health risks and the emergence of resistant pathogen strains. As a result, there is a pressing demand for sustainable alternatives in the management of *C. capsici* infections (11).

Bacteria that produce EPS and promote plant growth offer dual advantages of enhancing plant growth through the synthesis of hormones and improved nutrient absorption and suppressing plant pathogens by generating bioactive substances and creating protective biofilms. These bacteria are capable of solubilizing phosphate, fixing nitrogen and producing phytohormones like IAA and GA, which play direct role in promoting plant growth. Furthermore, certain EPS-producing bacteria produce biocontrol agents like siderophores, hydrogen cyanide (HCN) and lytic enzymes that suppress pathogens (12). Inoculation of EPS producing *Bacillus* spp. resulted in physiological alterations in maize seedlings that indicated the mitigation of drought stress. These included enhanced plant biomass, relative water content, leaf water potential, root-soil adhesion/root-tissue ratio, aggregate stability and reduced leaf water loss. Application of *Bacillus* spp. also led to a decrease in antioxidant enzymes activity, including ascorbate, peroxidase, catalase and glutathione peroxidase and increase in proline content under drought conditions. In the rhizosphere, EPS contributed to improving the soil's moisture retention capacity (3). Inoculation with growth-promoting rhizobacteria at 80 % field capacity of water regime led to an increase in plant biomass, attributed to a reduction in reactive oxygen species and an enhancement in antioxidant enzyme activity compared to the control (13). Many microbes, such as *Bacillus* spp. and *Pseudomonas* spp., are recognized for their EPS production, but further characterization and identification are required (14). The potential of EPS-producing bacterial consortia to promote plant health and suppress pathogens synergistically is particularly promising. These consortia exploit complementary traits among bacterial species to provide multifaceted benefits to plants. By improving soil structure and fostering beneficial microbial communities, such consortia represent a sustainable strategy for reducing chemical pesticide use and enhancing the resilience of chilli crops against anthracnose (15). In this study, we investigate the potential of bacteria that produce EPS to enhance plant growth and provide biocontrol benefits, with particular emphasis on *Colletotrichum capsici.*

Materials and Methods

Soil sample collection and isolation of exo-polysaccharide producing bacteria

Rhizosphere soil was collected in September 2024 from healthy chilli (*Capsicum annuum* L.) plants cultivated in an area under the Instructional Farm, College of Agriculture, Vellayani, where anthracnose disease was prevalent. EPS-producing bacteria were isolated from the soil using the serial dilution and spread plate technique on an EPS-selective agar medium composed of sucrose (40 g), $MgSO_4$ (0.2 g), KH_2PO_4 (3 g), K_2HPO_4 (9 g), yeast extract (2 g), agar (15 g) and distilled water (1000 mL) (16). A total of 42 bacterial isolates capable of producing EPS were obtained. Among them, three isolates exhibited both plant growth-promoting traits including IAA and GA production, siderophore production, phosphate solubilization, ammonia production and growth in NFB medium as well as antagonistic activity against *Colletotrichum capsici* and these were selected for the study. Consequently, molecular characterization was carried out for these three isolates.

Screening for plant growth-promoting traits

Indole-3-acetic acid (IAA) production

IAA was estimated using the method described by Gordon and Weber, 1951 (17). EPS-producing bacterial isolates were cultured for 3 days in nutrient broth with or without added tryptophan to assess IAA production in the presence and absence of the precursor respectively. To estimate IAA in presence of tryptophan, 1 mL of filter-sterilized (0.1 % w/v) tryptophan was added to 10 mL of nutrient broth and the isolates were inoculated. The culture broth containing tryptophan (0.1 % w/v) was incubated in the dark for 3 days. Following incubation, the culture was centrifuged at 3000 rpm for 30 min using Heraeus Megafuge 8R centrifuge (ThermoFisher Scientific) and 2 mL of the supernatant was transferred to a test tube. Two drops of orthophosphoric acid were added to the supernatant and allowed to stand for 1 min, after which 4 mL of freshly prepared Salkowski's reagent was added. The mixture was vortexed and incubated in the dark for 25 min. The colour changes from colourless to pale or dark pink was measured by recording the absorbance at 530 nm using a UV-Visible spectrophotometer (UV-1900i, Shimadzu). Blank was prepared using uninoculated broth by following the same procedure. The concentration of IAA in the sample was determined by comparing to a standard curve prepared with analytical grade IAA. The IAA production by the isolates were expressed in mg L^{-1}.

Gibberellic acid (GA) production

Gibberellic acid production was measured following the method described in a previous study (18). The isolates were cultured in nutrient broth for 4 days; cells were pelleted by centrifugation at 4500 rpm for 20 min in a Megafuge 8R centrifuge (Thermo Fisher Scientific). 15 mL of the supernatant was transferred to a clean centrifuge tube, to which 2 mL of zinc acetate reagent was added and incubated for 2 min. After incubation, 2 mL of potassium ferrocyanide was introduced and the mixture was centrifuged at 2000 rpm for 15 min. Subsequently, 5 mL of the supernatant was transferred to a test tube, followed by the addition of 5 mL of

30 % hydrochloric acid. The samples were incubated for 75 min at 20 °C in an incubator. Absorbance was measured at 254 nm using a UV-Visible spectrophotometer (UV-1900i, Shimadzu). Hydrochloric acid (5 %) was used as the blank. The concentration of GA within the specimen was determined by comparing it to a reference graph created using analytical grade GA. The results were expressed in mg L^{-1}.

Siderophore production

The isolates were tested for siderophore production using Blue Agar CAS medium, which contains chrome azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA) as indicators, following the method described by earlier researchers (19). The medium was prepared by adding 100 mL of MM9 salt solution (composed of 15 g KH_2PO_4, 25 g NaCl, 50 g NH_4Cl dissolved in 500 mL of distilled water) to 750 mL of distilled water. The pH was adjusted to 6, followed by the addition of 32.24 g piperazine-N,N'-bis (2-ethanesulfonic acid) (PIPES) and 1.5 % agar, after which the mixture was autoclaved. Once cooled to 50 °C, the medium was supplemented with 30 mL of a filter-sterilized 10 % casamino acid solution, 10 mL of a 20 % glucose solution and 100 mL of a dye solution. The dye solution was prepared by first dissolving 0.06 g of CAS in 50 mL of distilled water and mixing it with 9 mL of 0.0027 g $FeCl_3 \cdot 6H_2O$ dissolved in 10 mL of 10 mM HCl. This mixture was then combined with 0.073 g HDTMA dissolved in 40 mL of distilled water. The components were gently combined by adding the mixture along the glass wall with sufficient agitation to achieve a uniform blue colour. The prepared medium was aseptically poured into sterile plates and allowed to solidify. Bacterial isolates were inoculated onto the CAS plates and incubated at 28 °C for 24 hr. The formation of yellowish-orange halos around the colonies was considered indicative of siderophore production. The siderophore production index (SPI) was calculated using the formula:

SPI = (halo zone/colony diameter) × 100 (20).

Phosphate solubilization

Phosphate-solubilizing bacteria were isolated using NBRIP (National Botanical Research Institute's Phosphate growth medium) agar medium, with the following composition (g L^{-1}): glucose - 10.0, $Ca_3(PO_4)_2$ - 5.0, $MgCl_2.6H_2O$ - 5.0, $MgSO_4.7H_2O$ - 0.25, KCl - 0.2 and $(NH_4)_2SO_4$ - 0.1. The plates were incubated at 28 °C until transparent zones (halo zones) appeared around the bacterial colonies, typically within 2-3 days (12). The solubilization index (SI) was calculated after 7 days of incubation using the following formula.

SI = CD + HD/ CD

Where,

CD - colony diameter, HD - halo zone diameter (21).

Extracellular ammonia production

Extracellular ammonia production by the isolates was estimated following the method described in a previous study (22). The selected cultures were grown in peptone broth for four days. They were subsequently centrifuged at 4500 rpm for 20 min. 10 mL of the supernatant was transferred into a boiling tube and 1 mL of Nessler's reagent was added to it. The appearance of an orange to brown colour indicated the presence of ammonia in the supernatant. The quantification of ammonia was done by measuring the absorbance at 450 nm using UV-Vis spectrophotometer. Blank was prepared by the same procedure using uninoculated peptone broth. The extracellular ammonia production was expressed in mg L^{-1}.

Growth on N-free medium

The isolates were repeatedly sub-cultured on nitrogen-free malate bromothymol blue (N-free MBTB) medium to indirectly assess their capacity to fix atmospheric nitrogen. A loopful of culture grown in nutrient broth was spotted onto the N-free MBTB plates, which were then incubated at 28 ± 2 °C for 5-7 days. This process was repeated seven times to confirm the growth of the organism in N-free media. A positive result was indicated by bacterial growth accompanied by a colour change in the medium from green to blue, while the absence of growth and no colour change signified a negative result (23).

Amylase activity

Amylase production in bacterial cultures was evaluated using the starch hydrolysis test as described by a group of researchers (24). The chosen bacterial isolates were streaked onto starch agar plates and incubated at 28 ± 2 °C for 48 hr. Following incubation, the plates were flooded with Gram's iodine solution, which produced a deep blue colour due to the formation of starch-iodine complex. The presence of a clear zone around the bacterial growth, indicating starch degradation, was used to identify amylase-producing isolates.

Cellulase activity

The *in vitro* cellulase activity was assessed using carboxymethylcellulose (CMC) as a substrate. Pure bacterial cultures were streaked onto CMC agar plates and incubated for 72 hr. Following incubation, the plates were treated with an aqueous Congo red solution (1 mg/mL) for 15 min and then rinsed with 1 M NaCl. The presence of cellulolytic activity was confirmed by the appearance of clear zones around the bacterial colonies, indicating the degradation of CMC (25).

Quantification of exo-polysaccharide production

Exo-polysaccharide production of isolates was measured using a gravimetric method (26). The selected mucoid isolates were grown in sucrose amended EPS selective broth (100 mL) for 7 days. The broth was centrifuged at 2500 rpm for 20 min and the supernatant was carefully collected without disturbing the pellets. This supernatant was combined with chilled ethanol in 1:1 ratio and incubated at 4 °C for 24 hr. After incubation, the solution was centrifuged at 4500 rpm for 20 min and the resulting pellet was dried at 50 ± 2 °C for 3 days. The dried pellets were weighed to find the amount of EPS produced.

Antagonistic activity against *Colletotrichum capsici* in dual plate assay

The antagonistic potential of the EPS producing bacterial isolates against the chilli anthracnose pathogen, *Colletotrichum capsici*, was evaluated using dual plate assay. A uniform mycelial mat of *C. capsici* was obtained by inoculating the fungus to the centre of potato dextrose agar (PDA) plate and incubating at 28 °C for 5 days. Mycelial plugs were punched out of the fully grown *C. capsici* using a sterile cork-borer of 5 mm diameter. This mycelial plug was placed at the center of a PDA

plate and the bacterial isolates were streaked on both sides of the mycelial plug. Control plates were maintained by inoculating the mycelial plug of the fungus alone. The plates were incubated at 28 °C for 5 days and the inhibition of fungal growth was measured and the percent growth inhibition (PGI) was calculated as follows:

$$PGI\ (\%) = (KR - R1) / KR \times 100$$

where,

KR - distance (in mm) from the inoculation point to the colony margin on the control plates, R1 - distance (in mm) from the inoculation point to the colony margin on the treated plates in the direction of the antagonist (27).

The PGI was then classified on a growth inhibition category (GIC) scale from 0 to 4, where 0 = no growth inhibition; 1 = 1-25 % growth inhibition; 2 = 26-50 % growth inhibition; 3 = 51-75 % growth inhibition; 4 = 76-100 % growth inhibition. The inhibition zone was also recorded in mm after 7 days.

Identification of the exo-polysaccharide producing bacteria by 16S rRNA analysis

The molecular identification of purified EPS-producing bacterial isolates was conducted through 16S rRNA cataloging using universal primers (16S-RS-F: 5' CAGGCCTAACACATGCAAGTC3' and 16S-RSR: 5'GGGCGGWGTGTACAAGGC3'). Genomic DNA was extracted using the NucleoSpin® Tissue Kit (Macherey-Nagel, USA) following the method described in an earlier study (28). The quality and quantity of the isolated DNA were assessed via agarose gel electrophoresis and UV spectrophotometry. PCR amplification was performed in a 20 µL reaction mixture containing 1X PCR buffer (100 mM Tris HCl, pH 8.3; 500 mM KCl), 0.2 mM of each dNTP (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 1 unit of AmpliTaq Gold DNA polymerase, 0.1 mg mL^{-1} BSA, 0.7 µL of 4 % DMSO, 5 pM of forward and reverse primers and FTA disc derived DNA served as the template. The PCR was carried out using a GeneAmp PCR System 9700 (Applied Biosystems). The thermal cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 60 °C for 40 sec and extension at 72 °C for 60 sec, with a final extension at 72 °C for 7 min. Sequencing was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in accordance with the manufacturer's protocol, utilizing the GeneAmp PCR System 9700. Sequence quality was verified using Sequence Scanner Software v1 (Applied Biosystems). The contig sequence obtained after alignment using the BioEdit program (version 7.2.5) was analyzed with Nucleotide BLAST. The organism's identity was confirmed based on BLAST analysis.

Statistical analysis

Principal component analysis (PCA) was conducted to simplify the data's dimensions and to determine the key components that accounted for the highest variance among the traits. The analysis was conducted using GRAPES software (29). A biplot was generated to visualize the relationships between bacterial isolates and the different traits. The biplot represents principal components on the X and Y axes, trait vectors indicating their contribution to the components and bacterial isolates points to illustrate variation in the dataset.

Results and Discussion

The bacterial isolates producing EPS, derived from the rhizosphere of chilli (*Capsicum annuum* L.), demonstrated notable plant growth-promoting characteristics such as the synthesis of essential phytohormones that play a crucial role in boosting plant growth.

Phytohormone production

Plants react to drought stress by synthesizing IAA, a vital hormone for their adaptation to such conditions. Research has shown that a bacterial isolate's localized production of IAA in plant root nodules can modify the polar transport of IAA in the roots, promoting the development of more branched root systems, along with an increase in secondary roots and nodules (30). The bacterial isolates showed significant production of IAA, an essential auxin involved in plant growth and development. The IAA concentration was quantitatively measured after adding 0.1 % w/v tryptophan to the cultures. Among the tested isolates, *Bacillus halotolerans* CAJPH6 produced 28.7 µg mL^{-1} of IAA, followed by *Bacillus subtilis* CFORH5 with 28.3 µg mL^{-1} and *Bacillus stercoris* CAJRH6 with 26.5 µg mL^{-1} of IAA. Additionally, these EPS producing isolates were capable of synthesizing GA, a hormone involved in stem elongation and seed germination. *Bacillus halotolerans* CAJPH6 produced 0.32 mg mL^{-1} of GA, while *Bacillus subtilis* CFORH5 and *Bacillus stercoris* CAJRH6 yielded 0.31 mg mL^{-1} and 0.29 mL^{-1} respectively. Table 1 illustrates the plant growth-promoting characteristics of the EPS producing *Bacillus* isolates.

Phosphate solubilization

An earlier study suggests that EPS production in the rhizosphere can enhance water availability and nutrient levels, including phosphorus and potassium, while also aiding plants in coping with salinity (31). The EPS producing isolates from the chilli rhizosphere exhibited effective phosphate solubilization, with *Bacillus subtilis* CFORH5 achieving the highest phosphorus solubilizing index (PSI) of 1.7, followed by *Bacillus halotolerans* CAJPH6 with a PSI of 1.5

Table 1. Plant growth-promoting traits of EPS producing *Bacillus* isolates obtained from rhizosphere of chilli

Isolates	IAA (with trptophan) (µg mL^{-1})	IAA (without trptophan) (µg mL^{-1})	GA (mg mL^{-1})	PSI	SPI	Ammonia production (µmol mL^{-1})
CAJPH6	28.7	19.1	0.32	1.55	2.35	8.9
CAJRH6	26.5	22.1	0.29	1.65	1.65	8.78
CFORH5	28.3	21.7	0.31	1.7	3.0	9.1

IAA - Indole-3-acetic acid, GA - Gibberellic acid, PSI - phosphorus solubilizing index, SPI - siderophore producing index

and *Bacillus stercoris* CAJRH6 with a PSI of 1.45.

Siderophore production

The production of siderophores is a key mechanism in the biocontrol activity of various PGPR groups, including *Bacillus* sp., which produces a wide range of them. The current findings indicate that *Bacillus subtilis* CFORH5 had a siderophore production index (SPI) of 3.0, while *Bacillus halotolerans* CAJPH6 recorded an SPI of 2.3 and *Bacillus stercoris* CAJRH6 exhibited an SPI of 1.6, all isolated from the chilli rhizosphere. Previous study reported that siderophore-producing *Bacillus subtilis* LSBS2 significantly improved plant biomass, pigment content, iron levels and oil content in bioinoculant-treated sesame plants (32). This strain exhibited multiple plant growth-promoting (PGP) traits, including the production of HCN, ammonia and IAA, as well as phosphate solubilization. *Bacillus* spp. is recognized for its ability to enhance plant growth and protect against pathogen infections. Additionally, it plays a crucial role in mitigating biotic stresses by producing phytohormones, volatile organic compounds, EPS, siderophores and facilitating phosphate solubilization. Numerous studies on the use of various *Pseudomonas* species have shown that siderophores play a direct role in promoting plant growth and providing protection against different biotic stresses (33, 34).

Ammonia production

Ammonia was quantified using a spectrophotometric assay with Nessler's reagent, a strongly alkaline solution of potassium mercuric iodide (K_2HgI_4). In alkaline conditions, potassium, mercury and iodine interact in relation to ammonia concentration, forming a yellow brown to reddish-brown coloured complex (35). The reaction is as follow: $2K_2HgI_4 + NH_3 + 3KOH \rightarrow Hg_2OINH_2 + 7KI + 2H_2O$. The obtained complex, as indicated by its UV absorption spectrum, absorbs between 400 and 450 nm. Several studies have utilized this spectrophotometric method, with 450 nm being chosen as the optimal wavelength for quantifying ammonia production. It was found that 95 % of *Bacillus* spp., 94.2 % of *Rhizobium* spp., 74.2 % of *Pseudomonas* spp. and 45 % of *Azotobacter* spp. produced ammonia (36). Research indicates that *B. mojavensis* exhibited multiple plant growth-promoting rhizobacteria activities, including ammonia production, antifungal activity, HCN production and nitrogen-fixing ability (37). In our study, we observed that all three bacterial isolates tested were positive for ammonia production. The assessment of ammonia production showed that *Bacillus subtilis* CFORH5 generated 9.1 µmol mL^{-1}, followed by *Bacillus halotolerans* CAJPH6 with 8.9 µmol mL^{-1} and *Bacillus stercoris* CAJRH6 with 8.78 µmol mL^{-1}.

Growth on N-free medium

The ability of the isolates to thrive in the absence of combined nitrogen was assessed by repeated subculturing NFB medium, indicating their potential for atmospheric nitrogen fixation. However, this capability requires further confirmation through sequencing of *nif* genes. Growth on N-free medium along with other plant growth-promoting characteristics emphasize their ability to improve soil fertility and support sustainable agricultural practices. These results highlight the potential of these bacterial isolates as valuable bioinoculants for enhancing plant health and boosting agricultural productivity. Colony morphology of EPS producing bacterial isolates and its plant growth-promoting characteristics was presented in Fig. 1, 2.

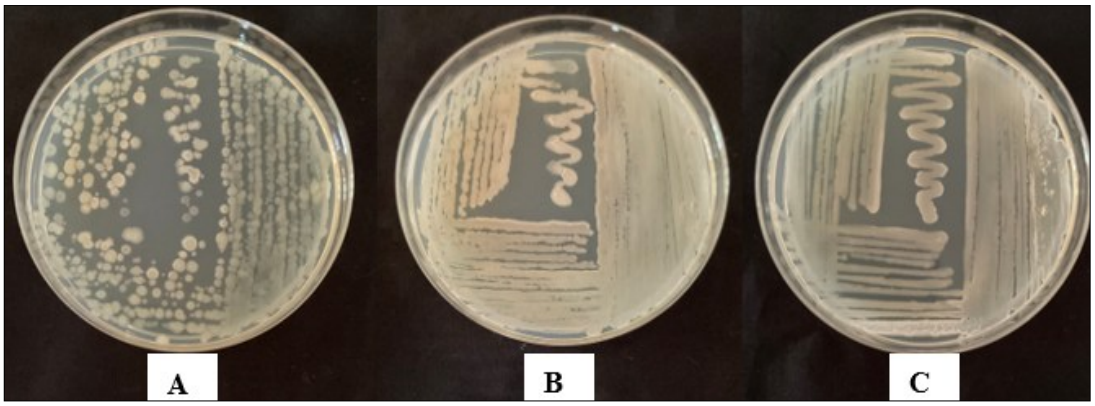

Fig. 1. Colony morphology of EPS producing bacterial isolates: A) *Bacillus halotolerans,* B) *Bacillus subtilis,* C) *Bacillus stercoris.*

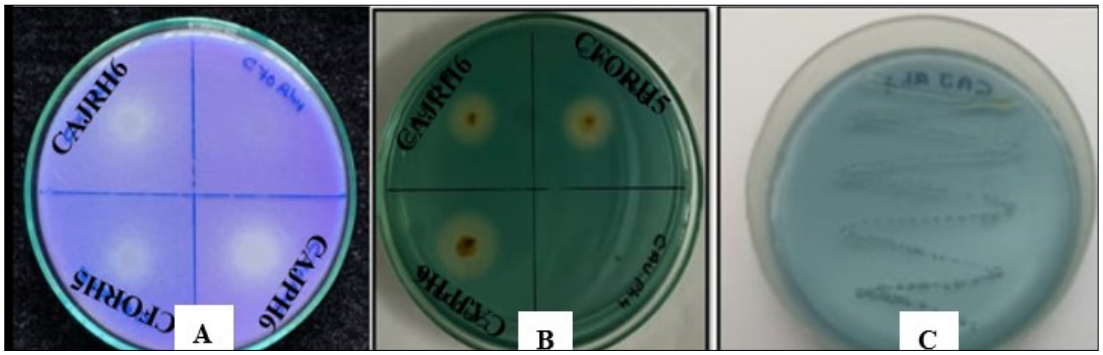

Fig. 2. Plant growth-promoting traits of bacterial isolates from chilli: A) phosphate solubilisation, B) siderophore production, C) presence of colony in nitrogen free medium.

Amylase and cellulase production

The rhizosphere bacterial community plays a crucial role in suppressing pathogens and promoting plant growth, either directly or indirectly, by producing plant growth regulators such as IAA, iron-chelating compounds and hydrolytic enzymes, including amylase, cellulase, pectinase, xylanase and lipase (38). In this study, the isolates CAJRH6, CFORH5 and CAJPH6 also demonstrated the ability to produce IAA, siderophores, ammonia and hydrolytic enzymes such as cellulase and amylase. The activity of amylase and cellulase was confirmed by the presence of halo zones around the inoculation site (Fig. 3, 4). Among various antagonistic mechanisms, cell wall degradation stands out as a key strategy employed by these biocontrol agents to efficiently manage fungal pathogens and enhance plant growth (39, 40).

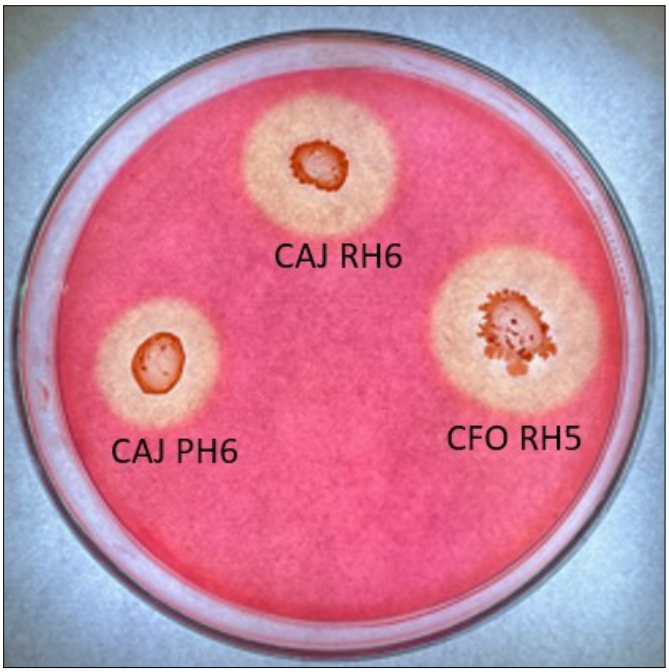

Fig. 3. *In vitro* evaluation of cellulase activity of EPS producing bacterial isolates: *Bacillus halotolerans* CAJ PH6, *Bacillus subtilis* CFO RH5 and *Bacillus stercoris* CAJ RH6.

Statistical analysis - Biplot

Principal component analysis biplot was performed to explore the relationships between variables and samples. In a biplot, arrows indicate the direction and strength of the loadings. The longer the arrow, the more significant the variable in that principal component. The angle between arrows shows how strongly variables are correlated. A small angle indicates a strong positive correlation, whereas a larger angle implies a weaker or negative correlation. Summary statistics of PCA are given in Table 2 and the corresponding biplot is shown in Fig. 5.

Quantification of EPS production

Researchers found that *Bacillus subtilis* and *Azospirillum brasilense* are capable of withstanding drought stress and individually produce significant amounts of EPS (41). When combined, these isolates produced higher levels of EPS (sugar 6976 µg g^{-1}, protein 731.5 µg g^{-1} and uronic acid 1.1 mg g^{-1}). The production of EPS provides microorganisms with a competitive advantage in drought-stressed environments. Lactic acid bacteria produced EPS ranging from 10 mg L^{-1} to 400 mg L^{-1} (42). In this experiment, gravimetric analysis revealed that *Bacillus stercoris* CAJRH6 yielded 0.93 mg mL^{-1} of EPS followed by *Bacillus subtilis* CFORH5 with 0.89 mg mL^{-1} and *Bacillus halotolerans* CAJPH6 with 0.72 mg mL^{-1}. EPS production is vital for biofilm formation, which boosts the bacteria's ability to establish themselves in the rhizosphere. These results emphasize the significance of EPS production by the isolates in promoting plant growth and contributing to a healthy, productive rhizosphere environment.

Antagonistic activity assessed by dual plate assay

Bacillus sp. is recognized for its ability to enhance plant growth and protect against pathogen infections (43). It also plays a crucial role in mitigating biotic stresses by producing

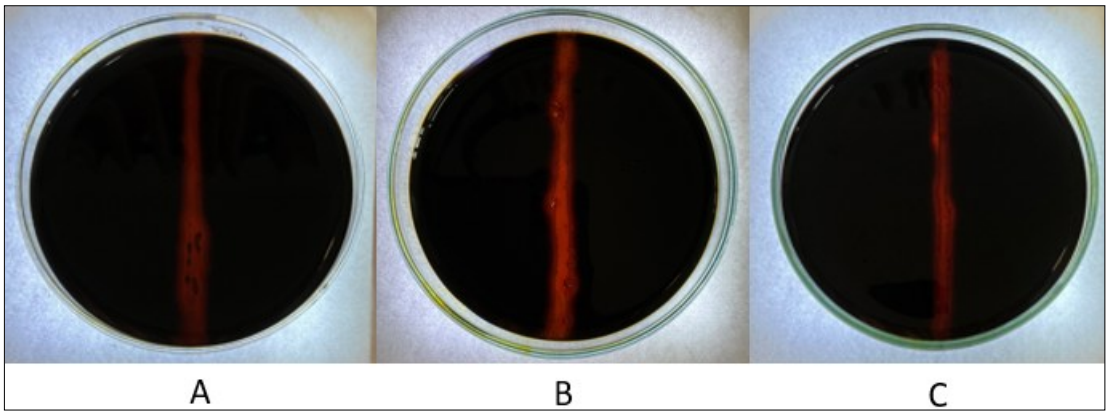

Fig. 4. *In vitro* evaluation of amylase activity of exopolysaccharides producing bacterial isolates: A) *Bacillus halotolerans,* B) *Bacillus subtilis,* C) *Bacillus stercoris.*

Table 2. NCBI-BLAST search results of 16S rRNA gene sequences of EPS producing bacterial isolates obtained from chilli rhizosphere

Isolate	Closest neighbour	Max score	Total score	Query cover %	E value	Identity %	Accession length	Accession number
CAJRH6	*Bacillus stercoris*	2307	27489	99 %	0.0	98.92 %	4282228	NZ_CP121861.1
CFORH5	*Bacillus subtilis* subsp. *subtilis*	2292	22806	100 %	0.0	98.47 %	4215606	NC_000964.3
CAJPH6	*Bacillus halotolerans*	1245	12457	100 %	0.0	98.59 %	4154245	NZ_CP029364.1

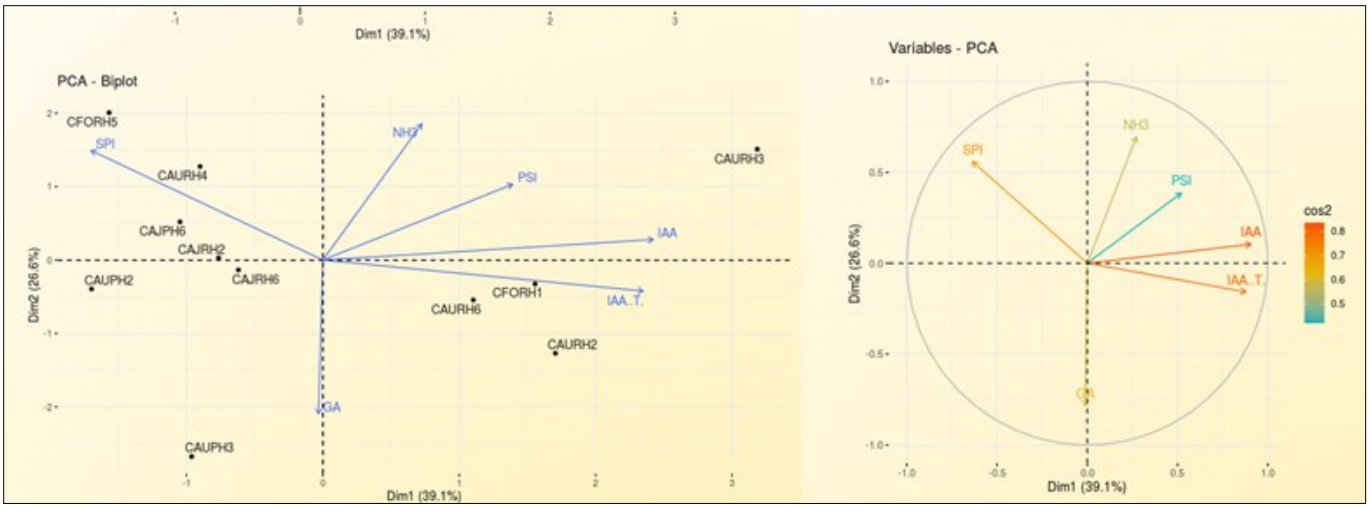

Fig. 5. Principal component analysis and biplot visualization of plant growth-promoting traits in *Bacillus* isolates.

phytohormones, volatile organic compounds, EPS, siderophores and by solubilizing phosphorus (44, 45). The application of *B. subtilis* has been shown to boost crop productivity. *Bacillus* species are considered among the most promising PGPR, offering an environmentally sustainable and cost-effective alternative to pesticide-based agricultural practices (44). Present study revealed that all three tested isolates exhibited significant inhibition of the pathogen. *Bacillus halotolerans* CAJPH6 demonstrated the highest inhibitory activity, achieving 72 % inhibition of *C. capsici* growth. *Bacillus stercoris* CAJRH6 closely followed this with 70 % inhibition and *Bacillus subtilis* CFORH5, showed a 64 % reduction in pathogen growth. These results suggest that these bacterial isolates have the potential to serve as effective biocontrol agents against anthracnose in chilli. Biocontrol of *C. capsici* using EPS producing *Bacillus* isolates is given in Fig. 6.

Molecular identification of isolates

The 16S rRNA method was used for the molecular identification of EPS producing isolates. Sequencing and comparison of their 16S rRNA gene sequences revealed that the identified isolates were *Bacillus stercoris* CAJRH6, *Bacillus subtilis* CFORH5 and *Bacillus halotolerans* CAJPH6. This technique is commonly employed to accurately identify bacterial species by analyzing the highly conserved regions of their ribosomal RNA genes. The NCBI-BLAST search results for the 16S rRNA gene sequences are presented in Table 2.

Conclusion

The microbial interventions, particularly the use of EPS producing bacteria are crucial for improving plant health and promoting growth. *Bacillus stercoris* CAJRH6, *Bacillus subtilis* CFORH5 and *Bacillus halotolerans* CAJPH6 were identified as the most effective and promising isolates, significantly secreting EPS. These bacterial isolates enhance nutrient availability and soil conditions through processes such as phosphate solubilization and siderophore production besides boosting plant resistance to biotic stress. The EPS produced by these microbes contributes to the formation of protective biofilms on plant roots, which can suppress harmful pathogens such as *Colletotrichum capsici*, a major threat to chilli plants.

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Authors' contributions

JPJB carried out experiments and performed the statistical analysis. JPJB and NC drafted the manuscript. NC, VIS, SAR and KNA conceptualized the study and contributed to its design. NC and VIS oversaw the coordination and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

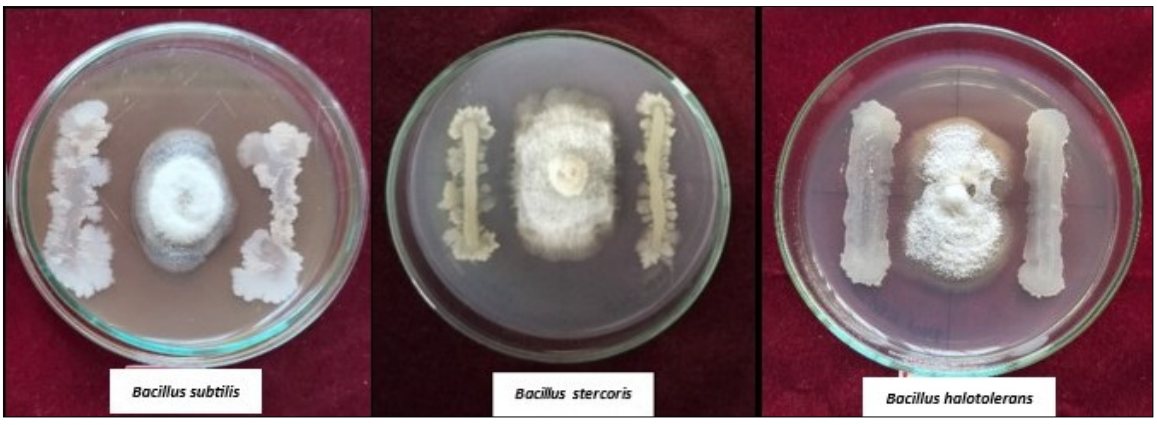

Fig. 6. Biocontrol of *Colletotrichum capsici* using EPS producing *Bacillus* isolated from chilli.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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