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Evaluation of Soybean-based Endophytic Bacterium *Pseudomonas moraviensis* PSSI3 for its Multifarious Plant Growth Promoting Potential in Soybean (*Glycine max* (L.) Merr.)

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ABSTRACT: Endophytic microorganisms that reside within plant tissues hold immense, yet largely untapped potential for enhancing plant growth and bolstering resilience against both biotic and abiotic stresses. In this study, twenty bacterial endophytes were retrieved from the soybean plants and underwent *in vitro* assessment for zinc solubilization and siderophores production. Among these strains, one isolate labeled as PSSI3 was identified as *Pseudomonas moraviensis* through 16S rRNA sequencing, exhibited the most significant growth-promoting qualities. PSSI3 endowed with multiple PGP attributes viz., zinc (8.76±0.17µg/mL), phosphorus (315±11.35 µg/mL), and potassium solubilization (315±11.35 µg/mL), siderophore production (63.06±2.29% unit), nitrogen fixation, HCN and ammonia production, biofilm formation, indole acetic acid (7.26±0.26 µg/mL) and gibberellic acid (20.45±0.73 µg/mL) synthesis. It also demonstrated biocontrol efficacy against fungal pathogens. Antibiotic sensitivity, carbon utilization, and tolerance to polyethylene glycol were examined. PSSI3 when introduced significantly enhanced soybean seedling growth, highlighting the potential of endophytes for impactful applications in crop production.

Keywords: Endophytes, Nutrient solubilization, Plant Growth Promotion, Soybean, *Pseudomonas moraviensis.*

INTRODUCTION

The agriculture industry has moved its attention to cutting-edge approaches like microbial inoculation to increase crop production in an environmentally sustainable manner, thereby reducing the ecological footprint associated with conventional practices such as the application of fertilizers and pesticides. This symbiotic link between plants and microbes not only improves agricultural characteristics, soil quality, and nutrient cycling but also offers a promising alternative to counteract the adverse impacts of traditional farming practices (Aktar *et al*., 2009; Singh *et al*., 2021). Plants offer a rich and complex macro-ecological environment teeming with microorganisms, serving as both hosts and nurturers for a wide array of inhabitants, including pathogens, symbiotic organisms, epiphytes, and endophytes. This relationship forms a hostile and dynamic equilibrium system. Endophytes, capable of entering plant tissues, particularly in vascular systems, cell gaps, xylem, and phloem, colonize plants systematically without causing harmful symptoms. The diverse array of plant structures provides an extensive range of niches for endophytic organisms, some of which possess the remarkable ability to stimulate plant growth and are commonly referred to as Plant Growth-Promoting Endophytes (PGPE) (Nair and Padmavathy 2014; Rahman *et al*., 2017).

Plant growth-promoting endophytes play a critical role in enhancing plant metabolic functions by initiating a diverse range of biochemical pathways. Their interactions with plants directly and indirectly stimulate plant growth, making bacterial endophytes indispensable for plant development. Direct mechanisms play a substantial role in the growth and differentiation of plant cells, tissues, and organs by producing phytohormones such IAA and GA_3 (Kamran *et al*., 2022). These endophytes boost nutrient solubility and exchange processes, encompassing the solubilization of phosphate (Varga *et al*., 2020), together with the solubilization of zinc and potassium. Furthermore, they supply nitrogen to the host plant and produce ammonia, thus ensuring the ready availability of these nutrients to foster plant growth (Singh *et al*. 2018; Qin *et al*., 2022). The uptake of nutrients in plants facilitated by rhizospheric microorganisms or endophyte inoculation involves various mechanisms. These include iron chelation by siderophores, alterations in soil pH due to organic acid secretion in root exudates, proton extrusion, and the production of phytohormones such as cytokinin, gibberellic acid, auxin, and ethylene (Chen *et al*., 2014). The production of siderophores enables the solubilization and sequestration of iron from the soil, thereby shielding plants from a variety of diseases (Maheshwari *et al*., 2019). Moreover, these endophytes play an active role

in protecting plants from environmental stresses, ultimately bolstering their ability to adapt (Rosenblueth and Martinez-Romero 2006). Indirect action refers to the process of mitigating the adverse effects of certain plant diseases on growth and yield. This involves the production of substances like antibiotics, HCN, biofilm, and extracellular polymeric substances (EPS) such as polysaccharides. These substances activate plant stress resistance and establish a harmonious symbiotic relationship with plants (Singh *et al*., 2020). The strategic utilization of beneficial plant endophytes in combating both biotic and abiotic stresses, including temperature, drought, salinity and nutrient deficiency, has gained significant attention in recent research (Kamran *et al*., 2022). Several studies reported the endophytic isolation from soybean and its characterization for their growth promoting activity (Kuklinsky *et al*., 2004; Vargas *et al*., 2019). A plethora of studies have explored the potential of plant growth-promoting endophytes (PGPE) like *Bacillus spp*, *Pseudomonas spp*, *Azospirillum*, *Burkholderia*, and *Pantoea* in enhancing the growth of various crops including rice, maize, wheat, soybean, tomato, sugarcane and green gram (Yadav *et al*., 2016; Vacheron *et al*., 2016; Rondeau *et al*., 2019; Kamran *et al*., 2022; Yadav *et al*., 2023; Amrutha *et al*., 2022). Soybean production faces numerous challenges, particularly due to diseases caused by fungal pathogens, including white mold, root rot, wilt and leaf spot caused by fungus *S. sclerotiorum*, *Fusarium oxysporum* and *A. alternate*. (Hosseini *et al*., 2023; Fagodiya *et al*., 2022). While the soybean anthracnose disease attributed to Colletotrichum species, causing issues like damping off, irregular spots, and acute damages of up to 100% (Boufleur *et al*., 2021).

Endophytic bacteria have been discovered within legume plants like alfalfa, clover, and pea. Various genera of bacteria have been identified in legume tissues, including *Aerobacter, Aeromonas, Agrobacterium, Bacillus, Chryseomonas, Curtobacterium, Enterobacter, Erwinia, Flavimonas, Pseudomonas,* and *Sphingomonas.* The diversity observed in legumes is remarkable due to their extensive cultivation history and adaptation to diverse agroclimatic conditions (Hung and Annapurna 2004). In the current study endophytic bacteria were isolated from soybean (*Glycine max* (L.)) a legume crop, rich in protein and oil content. The present study focal point is the isolation, molecular identification, and characterization of putative bacterial endophytes associated with soybean, specifically sourced from NEB CRC, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The study examined the qualitative and quantitative plant growth-promoting and biocontrol properties of these microbial isolates, assessing their direct impact on the growth of soybean seedlings. Despite the common occurrence of endophytes in numerous plants, there needs to be more information regarding Plant Growth-Promoting Endophytes (PGPE) and their functionalities, in stark contrast to the widespread prevalence of endophytes. A more profound understanding of native endophytes has the potential to unlock their capacity for enhancing plant growth and fostering sustainable crop production systems.

MATERIALS AND METHODS

Study area and sample collection: The roots, shoots and leaf samples of soybean were carefully gathered from NEB CRC, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (29° N and 79° E, elevation 243.8 m). The selected plant samples were gently uprooted and brought to the laboratory in sealed sterile polythene bags and stored at 4°C.

Isolation of endophytic bacterial isolates. Bacterial endophytes were isolated from soybean plant samples using various nutrient media (Nutrient agar, Actinomycetes agar, Pikovskaya agar, Pseudomonas agar, Aleksandrov agar) combinations. The samples (root, stem, and leaf) were surface sterilized using 70% alcohol for 1 minute, followed by sodium hypochlorite solution (4 %, 5 minutes for stem and leaf, 2 %, 10 minutes for roots); finally dipped in 70 per cent ethanol (30 seconds) and rinsed 6-8 times with sterilized distilled water. Samples were processed through two methods. In the first method, sliced samples were impregnated on different media and incubated at 28°C for 1 day, ensuring purity by using control plates (Manoharan *et al.*, 2016). The second method involved macerating 1 g of samples in 1 ml of distilled water, followed by seven-fold serial dilution, and pour plating. Plates were incubated at 28°C, and sterility was confirmed by checking for growth on control plates. Morphologically discrete bacterial colonies were purified through sub-culturing and preserved on slants at 4°C for routine uses and in glycerol stock at -20˚C for future investigation (Singh *et al*., 2017).

Screening for plant growth promoting traits through qualitative assay. Soybean endophytic isolates were assessed for plant growth stimulating traits such as zinc (Zn) solubilization in mineral salt medium supplemented with various insoluble zinc salts such as zinc oxide, zinc sulphate and zinc carbonate (Ramesh *et al*., 2014). Siderophore production using nutrient agar medium containing plates supplemented with chrome azurol S (CAS) dye solution. The presence of yellow-orange zone around the colonies is considered as positive result (Schwyn and Neilands 1987). Phosphate solubilization was examined using Pikovskaya agar plates following method given by Alkahtani *et al*. (2020) and potassium solubilization was performed by using Aleksandrow agar medium as described by Mursyida *et al*. (2015). In general, solubilization efficiency was calculated by dividing diameter of halo zone to the diameter of colony and multiplies by 100 to determine zinc, phosphorus, potassium and siderophore producing ability. Further, nitrogen fixation ability was determined by growth on Jensen's medium, a medium deficient in nitrogen (Kuswinanti *et al*., 2021). The capacity of endophytes to produce HCN was assessed using Miller and Higgins (1970). Filter paper soaked in alkaline picrate solution was sealed in petri plate containing endophytic bacteria on LB agar with 4.4 g L^{-1} glycine. Red-brown color change indicated HCN production by endophytes. Ammonia production was

evaluated by inoculating bacteria in peptone water and adding 0.5mL of Nessler's reagent. Positive samples exhibited a yellowish-brown hue, indicating ammonia generation (Cappuccino and Sherman 1992). Each of the assays mentioned was done in three replicates.

Screening for plant growth promoting traits through quantitative assay. The quantification of zinc solubilization was conducted following the procedure outlined by Fasim *et al*., (2002). The supernatant having soluble zinc was determined through Atomic Absorption Spectrophotometer (AAS). Furthermore, the pH of the filtered suspensions was monitored with a digital pH meter for accuracy and consistency. Quantification of siderophore production was achieved through the CAS-shuttle assay using the method of Payne (1994). The percentage (%) of siderophore produce in the aliquot was then determined. Quantitative assessment of phosphorous solubilized by bacterial isolates was performed in NBRIP (National Botanical Research Institute Phosphate Growth Medium) broth medium (Nautiyal, 1999) by adding Barton's reagent. The emergence of a yellow colour was noticed, and a spectrophotometer reading was recorded at 430 nm and pH changes were monitored. A standard curve of K_2HPO_4 concentrations was utilized to quantify solubilized phosphorus. IAA quantification in the culture filtrate was determined by method of Patten and Glick (2002) and expressed as μg mL^{-1} of the medium. Change of color to pink/red after mixing orthophosphoric acid and Salkowski's reagent indicated IAA production. GA_3 quantification in the culture was determined by method of Paleg (1965). EPS production was estimated by utilizing the method given by Siddikee *et al*. (2011). The ring formation approach was used to assess the test bacterial culture capacity to produce biofilms. Additionally, the amount of biofilm formed was calculated by dissolving a ring in 70 percent ethanol and measuring absorbance at 570 nm (Kotoky *et al*., 2019). Potassium solubilization was estimated by flame photometer after incubating isolates in Aleksandrow broth for 10 days (Sood *et al*., 2023).

Morphological and Biochemical Characterization. Endophytic bacterial isolates were further investigated for morphological and biochemical characterization since they showed promise as possible plant growth-promoting agents. The following morphological characteristics were examined in the current study: colony type, colony edge, elevation, colour, surface, and colony opacity (Boone *et al*., 2001). The Gram's reaction and different biochemical tests, including carbohydrate fermentation test, indole test, citrate test, catalase test (Aneja, 2006), MR-VP, gelatin test (Thomas *et al*., 2012), starch hydrolysis (Kasana *et al*., 2008), case in hydrolysis (Kasana *et al*., 2008), Cellulase activity (Hankin and Anagnostakis, 1975), urease production (Cappuccino and Sherman, 1992), lipolytic activity ((Kumar *et al.*, 2012), while endospore staining was done using Schaeffer Fulton method. Endophytes isolated from the samples underwent antibiotic sensitivity testing using discs purchased from Hi-media (OD258-1PK & OD020-1PK).

Polyethylene glycol (PEG) tolerant behavior. PEG tolerance by bacterial isolates was resolute by inoculating isolates in various concentrations of PEG (rangingfrom2%, 4%,6%, 8%,10%w/v) in tryptic soy broth and finally, tubes were kept for 24h of incubation at 28±2ºC. The bacterial strain that is capable of flourishing in liquid media supplemented with utmost concentration of PEG was mentioned as PEG tolerant endophyte (García *et al*., 2017).

Antibiosis activity against fungal pathogen

The antagonistic activity of the endophytic bacterial isolates against five fungal pathogens *viz*., *Fusarium oxysporum* f. sp. *Pisi*, *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium oxysporum* f. sp. *lycopersici, Sclerotinia sclerotiorum* were evaluated by dual culture assay (Dennis and Webster 1971) on PDA medium. Percent inhibition over control was carried out according to the formula given by Vincent (1947).

Spectrophotometric characterization of siderophore. The presence of hydroxamate siderophore was evaluated using the tetrazolium test, a method that capitalizes on the ability of hydroxamic acids to reduce tetrazolium salt when hydrolyzing the hydroxamate group in the presence of strong alkali, resulting in the appearance of a vivid crimson color (Snow, 1954). Additionally, hydroxamate siderophore production was proven using the $FeCl_3$ test, where positive results manifested as an orange color (Neiland, 1981). Catecholate siderophores were quantified using the Arnow (1937) method, while the identification of carboxylate siderophore was accomplished through the methodology established by Shenker *et al*. (1992).

Molecular characterization

Bacterial DNA was extracted using the CTAB method as described by Bazzicalupo and Fani (1995). DNA quality was assessed through electrophoresis on a 1% agarose gel, revealing a single band of high molecular weight DNA. Subsequently, a fragment of the 16S rRNA gene was amplified using 16S rRNA-F and 16S rRNA-R primers and the amplification was carried out in a PTC-200 thermal cycler. Consensus sequences of the 16S rDNA gene were generated by aligning forward and reverse sequence data using aligner software. The generated sequences were then compared to the GenBank database using the Nucleotide Basic Local Alignment Search Tool (BLAST N) program provided by the National Center for Biotechnology Information (NCBI). The sequences with the highest identity scores were selected, and the first 10 sequences were chosen. Sequence alignment was performed using the Clustal W package, and a phylogenetic analysis was constructed using the neighbor-joining method in MEGA software (Version 11). The confidence of the phylogenetic analysis was tested by bootstrap analysis with 1000 repeats. Finally, the sequences of the bacterial isolates were deposited in GenBank, and their accession number was obtained.

Plant growth promotion assessment

Preparation of substrates and seed bacterization. The substrate was prepared for the experiment by combining 10 kg of soil enriched with 2.5 kilograms vermicompost. This enriched substrate was then utilized for cultivating soybean seedlings in 25 separate cells. Seeds of soybean variety Pant soybean 1347 were

surface sterilized with 3 % sodium hypochlorite for 2 minutes, followed by ethanol (95%) for 1 minute and in 0.1% $HgCl_2$ for 3 minutes then rinsed in distilled water. Seeds were drenched in bacterial inoculums (10^8 cfu/ml) for 1 hr. The seeds which are soaked in sterilized distilled water are served as control. In an experimental period, 30 days after sowing (DAS), observations were noted on shoot and root length, number of primary branches, number of nodes, and plant dry weight.

Statistical analysis. Group differences were assessed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. Statistical significance was determined at $P \leq 0.05$ indicating significant difference. The analysis was accompanied using IBM SPSS 16 and Graph Pad Prism 9.

RESULTS AND DISCUSSION

Isolation of bacterial endophytes. Endophytes, which are microorganisms residing within discrete parts of plants, possess the remarkable capacity to foster plant growth and can function as bio-control agents, contributing to enhanced plant development. These endophytic microbes often engage in intricate interactions with their host plants, encompassing symbiotic, mutualistic, and various other types of relationships as highlighted by Fasusi *et al*. (2021). In this study, 20 unique bacterial endophytes displaying distinct morphologies were isolated using different media. Specifically, 7, 8, and 5 bacteria were extracted from soybean stems, leaves, and roots respectively, showcasing the diverse bacteria within different plant parts. Following the isolation and purification procedures, these bacterial isolates underwent screening based on their capabilities for siderophore synthesis and zinc solubilization. Subsequently, the best endophytic bacterial strain was selected, and their qualitative and quantitative PGP efficiency was further assessed.

Screening for plant growth promoting traits through qualitative assay. An assessment was undertaken to investigate the potential activities of bacterial endophytes related to plant development and health. The findings from this study revealed notable zinc solubilization efficiency by PSSI3 with values of 700% for ZnO, 375% for $ZnCO_3$, and 225% for $ZnSO_4$. Additionally, PSSI3 exhibited significant solubilizing efficiency for phosphate, siderophore, and potassium, with values of 566.7%, 211.2% and 250%, respectively. The isolates were also found to be positive for nitrogen fixation, HCN and ammonia production (Table 1). In previous research conducted by Upadhayay *et al.* (2022), it was reported that *Burkholderia cepacia* and *Pantoearodasii* demonstrated the competence to solubilized zinc and phosphate, produce siderophore, and ammonia, all of which promote the growth of paddy crops. Furthermore, *Pseudomonas moraviensis* has been shown to enhance wheat growth by solubilizing phosphate, making essential nutrients more readily available to the plants, as observed by Hassan and Bano (2016). In a prior investigation conducted by Shaikh and Saraf (2017), it was noted that *Exiguobacterium aurantiacum* exhibited Zn solubilization in a medium supplemented with ZnO and form a clear zone of 31 mm. Moreover, studies by Azizah *et al*. (2020); Qin *et al*. (2022) isolated endophytic bacteria with the capabilities of solubilizing phosphorus, potassium, and nitrogen fixation, thereby contributing to plant growth. Hydrogen cyanide (HCN), recognized for its biocontrol potential against plant pathogens, was generated by specific bacterial endophytes, as corroborated by Abdel *et al*. (2021), thereby contributing to enhanced plant growth. Another isolate, *Bacillus* sp., which was investigated by Gohil *et al*. (2022), exhibited plant growth-promoting capabilities, including ammonia production, indicating its potential for nitrogen fixation.

Screening for plant growth promoting traits through quantitative assay. The selected PSSI3 endophytic bacterial isolates underwent further assessment to quantify their potential for improving plant growth activities (Table 2, 3). Siderophores produced by microbes play a key role in promoting plant growth by facilitating iron uptake in plants and competing with harmful organisms for their availability. In the current study, PSSI3 displayed a notable siderophore production, measured at 63.06±2.29% unit. In a similar study, Maheshwari *et al.* (2019) isolated endophytes from the roots and nodules of chickpea and pea plants, discovering that the most effective endophytes produced siderophore exceeding 65% unit, promoting plant growth. Likewise, in the research conducted by Khan *et al*. (2023), it was observed that pseudomonas bacterial isolates demonstrated the capability to produce siderophores. This ability enabled them to render iron accessible for plants, and they also played a role in facilitating microbial-mediated iron accumulation in kidney bean grains. The bacterial isolate, PSSI3 also demonstrated its competence to solubilize phosphorus on the 3rd, 5th, and 7th days, with respective concentrations of 182.5±6.58µg/mL, 200±7.21 µg/mL and 315±11.35 µg/mL. The pH of cell-free supernatant on the seventh day of incubation was measured at 3.66±0.15. Phosphate solubilizing microorganisms (PSMs) play a critical role in mineralizing organic phosphorus, releasing inorganic phosphorus minerals, and storing a sizable amount of phosphorus in biomass, making it accessible to plants. In line with this, Khan *et al*. (2023) assessed the phosphate solubilization efficiency of *Pseudomonas jesenii* and *Pseudomonas palleroniana*, yielding values between 245.16 ± 8.6 µg/mL and 237.40 ± 6.7µg/mL under the NBRIB broth medium. While Singh *et al*., (2018) assessed the effectiveness of phosphate solubilizing *Chryseobacterium* sp. in promoting plant growth. They discovered that this bacterium exhibited the ability to solubilize tricalcium phosphate as 210.43 µg/mL. Further, PSSI3's zinc oxide solubilization potential was revealed to be 8.76±0.17 µg/mL through AAS analysis, accompanied by a reduction in pH to 6.0±0.29. Zinc deficiency in plants often results from insufficient zinc supply in an inaccessible form in the soil. Microorganisms employ various mechanisms, including the production of organic acids, siderophores, zinc chelation, and proton excretion to solubilize zinc, making it accessible to plants, as noted by Upadhayay

et al. (2019). The observed decrease in the pH of the liquid broth provides further evidence of the involvement of organic acids in zinc solubilization. In a similar vein, Bhatt and Maheshwari (2020) quantified the ZnO solubilizing capability of *Bacillus megaterium* through atomic absorption spectroscopy. A study by Khan *et al*. (2023) agrees with the present study, who also reported that the *Pseudomonas jesenii* MP1 bacterial isolate could solubilized zinc up to 18.50 ± 0.55 µg/mL with the decline in pH and further concluded the auxiliary role of organic acid production in Zn solubilization. Furthermore, PSSI3 demonstrated a significant ability to solubilize potassium, yielding a concentration of 91.69±3.30 ppm, coupled with a pH reduction to 5.8±0.91. Azizah *et al*. (2020) conducted a study and determined the potassium solubilization ability of endophytes isolated from maize crop and found a potassium solubilization ability of 8.38 µgmL^{-1}. Pérez-Pérez *et al*. (2021) emphasized the impact of pH reduction on the potassium solubilization process and its availability thereby promoting optimal nutrient uptake for plant development. Auxins, specifically Indole-3-acetic acid (IAA), and Gibberellic acid (GA_3) are vital phytohormones that act as essential regulators of plant growth. They are instrumental in initiating root formation and stimulating stem elongation. PSSI3 successfully detected the production of IAA at a concentration of 7.26±0.26 µg/mL and GA_3 at 20.45±0.73 µg/mL. In a study by Bhatt and Maheshwari (2020), *Bacillus megaterium* demonstrated the ability to produce 13.8 µg/mL of IAA. Additionally, Upadhayay *et al*. (2022) revealed IAA production ranging from 29.82 mg/mL in tryptophan-amended broth. Similar promising results were reported by Shylla *et al*., (2016), who isolated endophytic bacteria from an aerobic rice cultivar capable of producing GA_3. This discovery highlights the significant role of these phytohormones in enhancing plant growth and development. Although PSSI3 did not demonstrate extracellular polymeric substance (EPS) production, it exhibited biofilm formation with an optical density (O.D) of 0.71±0.025 at 570 nm. Biofilm production by endophytic Plant Growth-Promoting Bacteria plays a crucial role in their survival, especially under stressful conditions, as emphasized by Santoyo *et al*. (2016). Notably, bacterial isolates such as *Pseudomonas jesenii* and *Pseudomonas palleroniana* have been found to exhibit positive biofilm production. This ability facilitates soil aggregation, enhances effective colonization, and aids in nutrient uptake, as highlighted by Khan *et al*. (2023). These findings underscore the significance of biofilm formation in the ecological strategies employed by these bacteria for both survival and improved soil quality.

Identification of strain PSSI3. Strain PSSI3 was isolated from the stem of the soybean, and its genus and species were determined through a combination of phenotypic and molecular identification methods. The physiological and biochemical identification assessments confirmed that PSSI3 exhibited characteristics consistent with the *pseudomonas* genus (Table 4). PSSI3 displayed positive results for cellulase activity, lipolytic activity, citrate utilization and methyl red. Furthermore, it showed positive utilization of 17 different carbon sources. Sensitivity to specific antibiotics was determined by the occurrence of a clearance zone around the antibiotic disk, indicating susceptibility, whereas the absence of such a zone indicated resistance. Detailed data of the antibiotic sensitivity assay is presented in the Table 4. Further, strain PSSI3 was identified based on 16S rRNA sequencing, which led to its classification as *Pseudomonas moraviensis*. After obtaining the sequences, they were subjected to BLAST analysis using the NCBI database, which resulted in the assignment of the 16S rRNA gene sequence of strain PSSI3 to the accession number OR481732. Fig. 1 presents the phylogenetic tree and confirmed their taxonomic classification. It's worth noting that *Pseudomonas moraviensis*, as demonstrated in the current study, is well-established as a plant growth promoting and biocontrol agent in prior research. Staicu *et al*. (2015) identified *Pseudomonas moraviensis* as an endophyte that promotes the growth of *Brassica juncea*. Similarly, Cochard *et al*. (2022) evaluated the endophytic properties of *Pseudomonas moraviensis* showcasing its ability to enhance root growth in tomato plants.

PEG tolerance behavior. Isolate PSSI3 thrives only in nutrient broth supplemented with 2 % of PEG. Consequently, PSSI3 can be considered non- PEG tolerant endophyte.

Biocontrol activity. Plant growth-promoting bacterial endophytes serve a pivotal role in defending host plants against diseases through biocontrol mechanisms. They do so by either eliciting the plant's innate defense responses, such as induced systemic resistance (ISR), or through the production of an array of antibiotic chemicals and enzymes that actively counteract phytopathogens (Morales-Cedeño *et al.*, 2021). In this study, endophytic isolate PSSI3 recorded a remarkable inhibitory effect, recording inhibition rates of 40±0.89 %, 20±0.95%, 22.22±0.22 %, 40±0.10 %, and 45±1.44 % against five fungal pathogens i.e. *Fusarium oxysporum* f. sp. *Pisi*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotinia sclerotiorum*, respectively using the dual culture method (Fig. 2). Pseudomonas bacteria are renowned for their proficiency in colonizing plant roots and their ability to combat soil borne pathogens. Certain strains of Pseudomonas can even safeguard plants from leaf pathogens by triggering systemic resistance. Key mechanisms underlying the biocontrol capabilities of *Pseudomonas* agents include nutrient and space competition, antibiosis, and induced systemic resistance. Various Pseudomonas species, like *P. fluorescens, P. putida, P. protegens, P. syringae*, and *P. aeruginosa*, house these beneficial biocontrol strains (Vacheron *et al*., 2016). A bacterium known as *Burkholderia cepacia*, an endophyte extracted from mulberry leaves, demonstrates the ability to hinder the growth of the plant-pathogenic fungus *Colletotrichum dematium*, as reported by Ji *et al*. (2010). The current study also agreed with the study of Yadav *et al*. (2015), who observed that endophytes exhibited the ability to inhibit the mycelial growth of *F. oxysporum*. In the

present study, *Pseudomonas moraviensis* exhibited substantial disease-inhibiting potential and demonstrated remarkable efficiency in siderophore and hydrogen cyanide (HCN) production, both of which aid to its biocontrol properties. Wang *et al*., (2020) also highlighted the biocontrol potential of *Pseudomonas aeruginosa* against *Botrytis cinerea* and its ability to promote the growth of tomato plants. Similarly, Niem *et al*. (2023) demonstrated the biocontrol potential of endophytic *Pseudomonas poae* against *Neofusicoccum luteum*.

Spectrophotometric characterization of siderophore. Microorganisms exhibit remarkable diversity in synthesizing siderophores characterized by their distinct structural features and functional groups. These siderophores can be broadly classified into four types: catecholates, carboxylates, hydroxamates, and mixed type. Among these, bacteria primarily produce catecholates and carboxylates siderophores as their predominant siderophore types (Ahmed and Holmström 2014). The present study utilized spectrophotometric readings to recognize the chemical characteristics of siderophores. Notably, the PSSI3 isolate was found to be positive for hydroximate and carboxylate type siderophores. The findings indicate that the PSSI3 isolate possesses a rich array of functional groups within their chemical structure. In a study conducted by Kumar *et al*. (2021), the researchers successfully identified the specific type of siderophores produced by various *Escherichia coli* bacterial isolates, further underscoring the diversity in siderophore production within bacterial species. Aligning with our current research, Pattan *et al*. (2017) investigated siderophore-producing *Pseudomonas fluorescence* isolates and confirmed their capability to produce hydroxamate-type siderophores, thus corroborating the versatility of bacterial siderophore synthesis.

Growth Promotion in seedling trays. At the 30-day mark post-inoculation with strain PSSI3, we meticulously observed all growth parameters of soybean seedlings. The results showed a significant and favorable upswing in all growth parameters of soybean seedlings inoculated with strain PSSI3 in comparison to control. Key growth metrics, including shoot length (21.2±0.76 cm), root length (8.9±0.32 cm), number of primary branches (5±0.18), number of nodes (6±0.21) and dry weight (6.2±0.18) were markedly higher in the treatment (T2) than in the control (T1) (Table 5). These findings align with the work of Sharma *et al*., (2011), who also reported a significant positive effect on soybean growth following the inoculation of *Pseudomonas* spp. Similarly, Ramesh *et al*. (2014a) observed an increase in plant height of soybean when inoculated with *Bacillus aryabhattai.* In concurrence with our present study, Ramesh *et al*. (2014b) demonstrated that *Enterobacter cloacae* substantially enhanced shoot and seed weight of soybean at maturity. Furthermore, the inoculation of *Pseudomonas spp.* was found to increase the stem and root length, as well as the fresh and dry weight of soybean seedlings, leading to a significant boost in biomass compared to the control (Dubey *et al*., 2021). Singh *et al*. (2022) emphasized that the introduction of *Pseudomonas protegens* CP17 through inoculation led to a substantial enhancement in the germination, growth, and biomass of wheat seedlings. This improvement was notably significant when compared to the control group. In line with the research conducted by Joshi *et al*. (2018), an endophytic bacterium was isolated from *Ocimum sanctum* and *Aloe vera*, effectively enhanced the growth of green gram seedling.

Table 1: Qualitative plant growth promoting efficiency of endophytic bacterial strain PSSI3.

Bacterial Isolate	% zinc solubilization efficiency (ZnO)	% zinc solubilization efficiency ($ZnCO_3$)	% zinc solubilization efficiency ($ZnSO_4$)	% phosphate solubilization efficiency	Siderophore production efficiency	% Potassium solubilization efficiency	Nitrogen fixation	Ammonia production	HCN Production
PSSI 3	700	375	225	566.7	211.2	250	+++	+	+

Table 2: Quantitative estimation of plant growth promoting properties of endophytic bacteria.

Bacterial Isolates	IAA production (µg/mL)	GA_3 Production (µg/mL)	% Siderophore unit	EPS production (mg/mL)	Biofilm Production
PSSI3	7.26±0.26	20.45±0.73	63.06±2.29	0.00	0.71±0.025

Mean±SE is shown in the table; each value is the mean of three replicates

Table 3: Quantitative analysis of zinc, potassium and phosphorus solubilization of endophytic bacterial strain PSSI3.

Isolates	Zinc oxide solubilization		Potassium solubilization		Phosphorus solubilization (µg/mL)			
	(µg/mL)	pH	(ppm)	pH	3rd DOI	5th DOI	7th DOI	pH
PSSI3	8.76±0.17	5.96±0.19	91.69±3.30	5.4±0.94	182.5±6.58	200±7.21	315±11.35	3.66±0.15

Mean±SE is shown in the table; each value is the mean of three replicates

Table 4: Morphological, Biochemical, Carbohydrate utilization and Antibiotic sensitivity test of PSSI3.

Morphological and Biochemical characteristic		Carbohydrate utilization				Antibiotic sensitivity	
Characteristics	PSSI3	Carbon sources	PSSI3	Carbon sources	PSSI3	Antibiotic (µg/disc)	PSSI3
Gram reaction	Gram negative	Lactose	Positive	Sorbitol	Negative	Cephalothin (CEP, 30 µg/disc)	-
Shape	Circular	Xylose	Positive	Mannitol	Positive	Clindamycin (CD, 2 µg/disc)	-
Edge	Even	Maltose	Positive	Adonitol	Negative	Co-Trimoxazole (CO, 25 µg/disc)	-
Elevation	Convex	Fructose	Negative	Arabitol	Positive	Erythromycin (E, 15 µg/disc)	+
Surface	Slightly glossy	Dextrose	Positive	Erythritol	Negative	Gentamicin (GEN, 10 µg/disc)	+
Chromogenesis	Yellowish white	Galactose	Positive	α-methyl-D-glucoside	Negative	Ofloxacin (OF, 1 µg/disc)	+
Endospore	_	Raffinose	Negative	Rhamnose	Positive	Penicillin-G (P, 10 µg/disc)	+
Starch hydrolysis	_	Trehalose	Negative	Cellobiose	Positive	Vancomycin (VA, 30 µg/disc)	-
Urease Production	–	Melibiose	Negative	Melizitose	Negative	Chloramphenicol (C, 30 µg/disc)	+
Catalase Production	–	Sucrose	Positive	α-methyl-D-mannoside	Positive	Ampicillin (A, 30 µg/disc	-
Gelatinase Production	–	L-arabinose	Positive	Xylitol	Negative	Tetracycline (T, 15 µg/disc	-
Casein Hydrolysis	_	Mannose	Positive	ONPG	Positive	Kanamycin (K, 30 µg/disc	+
Cellulase Activity	+	Inulin	Positive	Esculin	Negative	Amikacin (AK, 15 µg/disc	+
Lipolytic Activity	+	Sodium gluconate	Positive	D-arabinose	Negative	Streptomycin (S, 15 µg/disc	+
Citrate Utilization	+	Glycerol	Positive	Citrate	Negative	Polymixin B (PB, 10 µg/disc)	-
Indole	_	Salicin	Negative	Malonate	Negative	Cefuroxime (CXM, 15 µg/disc)	-
Methyl red	+	Dulcitol	Negative	Sorbose	Negative	Erythromycin (E, 15 µg/disc)	+
Voges proskauers	–	Inositol	Negative	-	-	Cloxacillin (COX, 10 µg/disc)	+

Note: In molecular and Biochemical characterization + sign indicate presence of activity and – indicate absence of activity. In antibiotic sensitivity + Sign represents presence of sensitivity against antibiotic, whereas – sign represents resistance.

Table 5: Effect of potential endophyte on agronomical parameters of soybean seedlings.

Treatments	Shoot length (cm)	Root length (cm)	Number of primary branches	Number of nodes	Dry weight (g/plant)
T1(Control)	15.2±0.66	5.4±0.19	4±0.14	5±0.18	3.9±0.14
T2 (PSSI3)	21.2±0.76	8.9±0.32	5±0.18	6±0.21	6.2±0.18

Data were analyzed at P<0.05 level of significance. Mean±SE is shown in the table; each value is the mean of three replicates

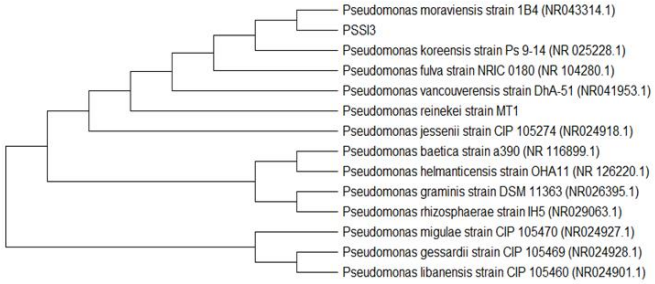

Fig. 1. Phylogenetic tree of PSSI3 endophytic bacterial strain constructed through MEGA.

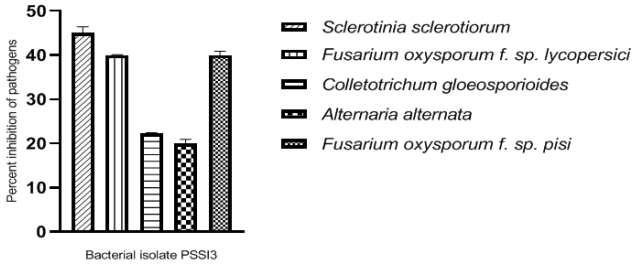

Fig. 2. Biocontrol activity against fungal pathogens.

CONCLUSIONS

This comprehensive investigation sheds light on the multifaceted mechanisms employed by *Pseudomonas moraviensis* PSSI3 to foster the growth of soybean seedlings. By delving into both direct and indirect pathways, this research illuminates the intricate ways in which endophytic bacteria, particularly PSSI3, can significantly enhance the productivity and health of plants. Moreover, the utilization of such beneficial endophytes not only augments plant growth but also plays a pivotal role in enhancing soil quality and fertility.

FUTURE SCOPE

Comprehensive evaluation of *Pseudomonas moraviensis* strain PSSI3 growth-promoting efficiency at the field trials should be conducted to assess its impact on soil health, fertility, and nutrient status, particularly focusing on soybean grain quality. Understanding its influence on overall crop yield, nutrient uptake, and soil ecosystem dynamics will contribute valuable insights for sustainable agricultural practices, through the harnessing of *P. moraviensis* as a potent biofertilizer.

Author Contributions. VB performed research, analyzed data and drafted manuscript. BK assisted in carrying out the research work. TS and AK were responsible for editing the manuscript. AVS provided guidance for the finalization of the manuscript and the research process.
All authors contributed to the article and approved the submitted version.

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Conflict of Interest. None.

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