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INVESTIGATING THE EFFICIENCY OF ENDOPHYTIC BACTERIA FROM *FAGONIA INDICA* IN PLANT GROWTH PROMOTION

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Abstract

The application of endophytic bacteria within agriculture demonstrates significant potential in promoting sustainable agriculture and potential alternatives to pesticides and chemical fertilizers. The microbial approach is increasing accessibility of nutrients for plants that is crucial for sustainable agriculture and other farming practices. The current study was performed to focus on isolation, molecular identification and assessment of plant growth promoting traits of endophytic bacteria from *Fagonia indica* roots. Four bacterial strains, *Siccibacter colletis*, *Enterobacter cloacae*, *Pseudomonas canadensis* and *Staphylococcus warneri* coded as MI-1, MI-2, MI-3, and MI-4 strains were identified and assessed for plant growth promoting assays such as siderophores production, phosphate solubilization, IAA production and extracellular enzyme activities along with HCN and antifungal activity under *İn vitro* conditions. These bacteria were also characterized morphologically and biochemically. The results revealed that MI-4 was identified as Gram-positive, whereas the others were Gram-negative and biological aspects demonstrated multiple plant growth-promoting traits. All the bacterial strains were efficient phosphate solubilizers, MI-4 produced siderophores, all strains showed positive results for IAA, ammonia and HCN production. Antifungal activity against *Rhizoctonia solani* and *Aspergillus niger* was exhibited by all the selected bacterial strains. Enzyme assays confirmed pectinase and protease activities. Application of endophytic bacterial strains on *Triticum aestivum* significantly increases root and shoot growth *İn vitro*. This research emphasizes the ability of endophytic bacteria role as biofertilizers and biocontrol agents, promoting and improving the sustainable agricultural practices by increasing the plant growth and reducing the attack of plant pathogens.

Key words: Endophytic bacteria, Plant growth promoting traits, *Fagonia indica*, *Triticum aestivum*, Sustainable agriculture

Introduction

Approximately 5.2 billion hectares of agriculture land are affected by salinity, soil degradation, and erosion (Vaishnav and Patel, 2023). Our fertile lands are disturbing due to salinity stress considerably therefore, country economy and agriculture are affected badly because the abiotic stress such as salinity stress has worst effects on the plants growth and development as well as dropping yield (Ullah *et al*., 2025). The architecture of plants holds specific mechanisms for the mitigation of salinity stress, including hormones stimulation, exchange of ion, activation of signalling flows on their metabolic, antioxidant enzymes and genetic frontiers that quite the stressed situation. In addition to plant natural mechanisms, certain microorganisms also contain specialized mechanisms that have ability to plant growth promotion and salt stress tolerance. "Endophytes" is a Greek-derived term that signifies "within the plants." The application of this term is as expansive as its literal definition, covering a potential hosts and inhabitants such as bacteria, fungi, plants and sometime the insects inside the plants and algae (Schulz & Boyle, 2006).

PGPB increases the uptake of nutrient contents, plant growth, resist to biotic stresses i.e. pathogen suppression and abiotic resilience by reducing dependency on chemical inputs, in this manner promoting sustainability of agriculture and food security (Ramakrishna *et al*., 2019; Maqbool *et al*., 2025; Khan *et al.,* 2026). These are the symptomless bacterial and fungal microbes present in all known plant species. These plant-associated microbes establish symbiotic relationships with their host plants by inhabiting internal tissues. This unique association reduces them essential in agriculture, serving as a potent tool for enhancing crop productivity (Dutta *et al*., 2014). These bacteria also trigger plants to produce different plant growth hormones like cytokinins, gibberellin and auxin, as well as volatile organic compounds (VOC). Additionally, these bacteria produce regulators of plant growth like siderophores involved in nitrogen fixation, solubilizing both the organic and inorganic phosphate. The different mechanisms and aspects of bacteria play significant role in promotion of plants growth and yield and can also be used as a cost effective and economical tool to control plant diseases (Numan *et al*., 2018; Yadav, 2020a). Sustainable agricultural practices are important for mitigation of environmental impact, increasing resilience against climate change, and ensuring long-term economic viability for

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farmers worldwide (Khan *et al*., 2025). These practices indorse the conservation of biodiversity, soil health, resource and community livelihoods, promoting an approach to balance the food production that meets present needs without compromising future generations (Safruddin *et al*., 2024).

Endophytes possess plant growth-promoting (PGP) attributes, are now recognized as crucial components for sustainable agriculture. These PGP microbes play an important role in increasing the plant growth, whether through direct mechanisms like the release of plant growth regulators, phosphorus, potassium, and zinc solubilization, biological nitrogen fixation, or via the production of siderophores, ammonia and other secondary metabolites that act antagonistically against pathogenic microbes (Yadav, 2020b).

Bacterial endophytes widely inhabit the internal tissues of plants, promoting the range of interactions such as commensalistic, symbiotic, trophobiotic, and mutualistic relationships (Khan *et al*, 2020). Majority of the endophytic bacteria have been characterized from most medicinal plants, owing to their extensive biosynthetic capabilities (Aswani *et al.*, 2020). Bacterial endophytes protect their host plant often from abiotic stresses such as drought state, increased pollution or salinity and against herbivores and pathogens (Papik *et al*., 2020). Endophytic bacteria are crucial in promoting plant growth through a range of indirect mechanisms, which involve the control of pests and pathogens via hydrolytic enzymes, antibiotics, biocontrol capabilities, and nutrient competition with pathogens (Prasad *et al*., 2020). Bacterial endophytes also have been reported to increase the growth of plant through various biochemical and physiological attributes, including siderophores production, phosphate solubilization, biological fixation of nitrogen, and the synthesis of plant-growth-promoting substances (Eid *et al*., 2021).

Endophytic bacteria have significantly an increasing effect on the growth of plants by producing phytohormones, biofertilizers, and aiding stress tolerance that offers a promising application in agriculture and biotechnology. Similarly, their ability to strengthen plant immunity and produce novel compounds suggests potential for disease control and environmental remediation, showcasing their versatility and broad spectrum of applications (Adeleke *et al*., 2021). Endophytes enhance plant growth via various activities, including facilitating nutrient uptake (such as nitrogen fixation, phosphate solubilization, and iron absorption), synthesizing hormones (such as auxin, gibberellins, ethylene, and cytokinins), bolstering disease resistance or biocontrol via siderophores production, HCN production, antibiosis, and hydrolytic enzymes production), and increasing stress tolerance (Farheen *et al*., 2016; Khan *et al.,* 2025).

Fagonia indica is a significant member of the *Fagonia* genus that is a thorny herb reaching an approximate height of 60 cm and width of 100 cm (Rahman *et al*., 2017). It belongs to the Zygophyllaceae family, which encompasses nearly 22 genera and over 250 species, distributed across various phytogeographical regions globally. *Fagonia indica*, commonly referred to as Sachi booti, Dhamana, Dhamasa, and Shoka'a, thrives in desert climates of Africa and Asia, adapting to diverse environmental conditions (Puri and Bhandari, 2014). *Fagonia indica* is traditionally employed for treating diverse ailments like fever, asthma, urinary discharges, toothache, stomach troubles, and kidney diseases, which contain various bioactive compounds including saponins, sterols, alkaloids, flavonoids, terpenoids, coumarins, amino acids, potentially contributing to its medicinal efficacy (Ali & Khan, 2021). *F. indica* is a very important medicinal plant because it has different therapeutic and traditional uses such as anti-inflammatory, antipyretic, anti-leishmanial, anticancer, antidiabetic, laxative, gastro protective, hepatoprotective and antioxidant effects (Sengupta & Gunri, 2015). Limited studies exist on the endophytic bacteria of *F. indica* and their role in plant growth promotion.

This study aimed to isolate, molecularly characterize, and evaluate the efficiency of endophytic bacterial strains from *Fagonia indica* in promoting plant growth under *In vitro* conditions. The specific objectives were to identify the plant growth-promoting traits of the isolated strains, including siderophore production, phosphate solubilization, indole acetic acid synthesis, and antifungal activity. Additionally, to assess the potential of these strains in enhancing root and shoot growth of *Triticum aestivum* (wheat). Moreover, to explore their possible application as biofertilizers for sustainable agriculture under stress conditions. This research provides potential microbial based solutions to challenges posed by climate change and the excessive use of chemical fertilizers.

Materials and Methods

The current research was carried out in Molecular Systematics and Applied Ethnobotany Lab (MoSAEL), Department of Plant Sciences, Quaid-i-Azam University Islamabad. The experimental work covers isolation of isolation of endophytic bacteria from *Fagonia indica*, their identification through 16S rRNA gene sequence analysis, morphological identification, biochemical testing, and assessment for plant growth promotion. The plant material was collected from village Mulazai, Peshawar, Pakistan (71.47318° E longitude and 34.05161° N latitude). This region is native habitat for *F. indica* having altitude of about 300-350 meters above sea level falling within the region of semi-arid to arid climatic. Additionally, this area experiences a semi-arid climate which was characterized by mild winters and hot summers and sparse rainfall particularly occurs during the monsoon season. The type of soil was predominantly sandy and clayey appropriate for desert and semi-desert plants as *F. indica*. It was surrounded by *Acacia modesta, Calotropis procera, Cymbopogon jawarancusa etc.*

Isolation of endophytic bacteria and Molecular characterization: The roots of *Fagonia indica* were first cleaned through tap water to remove mud, then sterilized with 70% ethanol for 1 min, and washed with autoclaved water to ensure sterilization before placing the root cut into segments on TSA medium for isolation of endophytic bacteria. The plates were incubated for 24 hours at 28°C to

allow colonies formation. The distinct colonies were selected, isolated on the basis of morphology including shape, color etc and preserved in glycerol at -80°C deposited under strains codes of MOSEL-FIL 16, MOSEL-FIL 18, MOSEL-FIL 20, and MOSEL-FIL 24, respectively. This method aims on isolating endophytic bacteria while excludes microbial community and analyses of physical chemical in soil, which are non-relevant to the scope of this work.

The isolated endophytic bacteria were molecular identified by extracting DNA using the plain boiling method as described by (Rahman *et al*., 2017). The gene of 16S rRNA was amplified through PCR with 27F (5′-CAGAGTTTGATCCTGGCT-3′) and 1492R (5′-AGGAGGTGATCCAGCCGCA-3′) universal bacterial primers, producing a 1465-bp product. The PCR products were purified by Sanger sequenced at Microgen in South Korea and compared the sequences with GenBank database. Complete lengths of 16S rRNA genes sequences were uploaded in GenBank with accession numbers OP727567, OP727568, OP727569, and OP727570.

Morphological identification of endophytic bacteria: Using a microscope, the colony morphology of four endophytic bacteria was examined. Physical features noted include size, form, texture, margins, and colour of the colony of four above mentioned bacteria. Gram's staining was the standard method used to perform this.

Gram's staining: A morphological technique, familiar to designate bacteria species into two paramount: Gram positive and Gram negative by assessing the physical and chemical attributes of their cell walls. Gram staining was performed as follows: preparation of microscopic slide was carried out as; a water droplet was positioned on glass slide, and a little quantity of bacterial culture was suspended in it to attain the stain of bacterial colony. Bacterial culture was dried in air. A few drops of crystal violet stain were poured on it and let for drying for 60 seconds, removal of excess stain was done by gentle washing with distilled water. Then flooding the slide with Gram's iodine was done and let it for drying for 60 seconds. Gentle rinsing of the slide with distilled water was performed. A few drops of 70% ethanol (decolourizer) were added until the slide is free of stain colour. Safranin was added for counter staining for 40-45 seconds, which was then washed with water and allowed to dry air. After one-hour slide was observed under compounds microscope (Parasuraman *et al*., 2024).

Biochemical test for identification of endophytic bacteria

Methyl red test: The ability of bacterial endophytes to stabilize high amounts of organic acid was determined using the methyl red test. After preparation, the MR broth medium was autoclaved and then transferred to sterile test tube (15 mL in each). Using an inoculating loop, bacterial cultures were inoculated in TSB media and maintained at 37ºC for 24 to 48 hours in a shaking incubator. After incubation, color change was seen after addition of few drops of methyl red indicator (Van *et al*., 2019).

Citrate utilization test: Simmon citrate agar is a well-defined media comprising of sodium citrate as the source of carbon and ammonium ion which is only nitrogen source. The media was prepared, autoclaved, and poured into sterilized test tubes and solidified by keeping the test tubes in tilted position. Strains of fresh bacteria were spotted on media using sterilized loop and then incubated at 37°C for 24 hours. Bacteria's utilization of citrate for its metabolism is changing media from frost green to royal blue color, which shows positive results. The change in media color from frost green to royal blue was observed as a positive result, indicating that the bacteria utilized citrate for its metabolism (Salo & Novero, 2020).

Mac-Conkey agar test: Mac-Conkey agar medium can be used to identify lactose fermenters and is selective for gram-negative bacteria. After preparation in distilled water, the media was autoclaved. It was poured and allowed to solidify in sterile petri plates. On this medium, the strains of bacteria were streaked. With the aid of a heat-sterilized loop, the strains of bacteria were spread on the medium, then it was incubated at 37°C. After incubation for 24-48 hours, the findings were recorded. The medium will turn pink due to the bacteria's capacity to ferment lactose (Taneja *et al*., 2023).

Urease agar: To determine whether bacteria could break down urea by creating the enzyme urease, the Christensen urea agar media was employed. It was prepared, autoclaved, poured into test tubes that had been autoclaved, and allowed to solidify. Using a heat-sterilized loop, fresh bacterial strains were streaked onto the medium and then incubated for 24-48 hours at 37°C. Positive outcomes are indicated by the appearance of pink color (Pundir *et al*., 2014).

Biological evaluation of endophytic bacteria for plant growth promotion

The plant growth promotion abilities of selected bacterial isolates were checked with the help of the different assay's characteristic of this trait in plant growth promoting bacteria.

Phosphate solubilization assay: Phosphate solubilization activity of bacteria was determined by using the National Botanical Research Institute Phosphate (NBRIP) medium, with addition of tri calcium phosphate. After preparation and solidification of media bacteria were spot inoculated on the media. Then plates were incubated for 1 week at 37°C and zones formation was observed. Formation of zones indicated positive results, zones size was assessed using vernier caliper (Younis *et al*., 2024a).

Siderophores production: Schwyn and Neilands, 1987 proposed a modified protocol to find out production of siderophores on media i.e., CAS agar. Solution containing iron complexed CAS dye, named Solution-I, is an indicator. To stabilize the complex of iron with CAS solution 40mL HDTMA was added. Buffer solution,

named as solution-II equipped in 750 mL of distilled water by adding several components, for adjusting pH 50% potassium hydroxide was added. Then Solution-II, a buffer, was made in 750 mL sterile water by adding components and adjusting pH with 50% KOH. Agar (15g) was added to reach 800mL. Solution-III was prepared in 70mL distilled water. Solution IV was 10% casamino acid. All autoclaved separately, cooled to 50°C. Mixed: III into II, then 30mL of IV. Solution-I added carefully, agar added. Poured into petri plates, solidified in laminar flow hood. After bacterial inoculation, it was incubated at 37°C for a week. Orange halos observed daily (Sun *et al*., 2024).

Indole acetic acid production: Fresh bacterial cultures were introduced into TSA medium, both with and without the addition of tryptophan. For 24 to 48 hours, cultures were incubated at 37°C in a shaking incubator. Tryptophan was added to the medium in an amount of 500 µg/mL, and the inoculum, which was prepared as a cell suspension, was given in a volume of 50 µL. Following a 24-48 hours incubation period, 1 mL of the bacterial culture was taken in an Eppendorf tube and centrifuged at 1000 rpm for 5 minutes. Separately, the supernatant was placed in a tiny test tube and left for 25 minutes and treated with 2 mL of Salkowski's reagent. The formation of indole acetic acid is indicated by the solution turning pink. Using a UV Spectrophotometer, the absorbance of the solution was measured at 530 nm after 25 minutes. Tryptic soya broth (TSB) was used as a control. Indole Acetic Acid (IAA) shows color pink in the presence of $FeCl_3$ (Younis *et al*., 2024b).

Ammonia production: Peptone water is used to grow bacteria for checking their ammonia production ability. 10 mL of peptone water is taken in each test tube, fresh bacterial culture was inoculated in them and incubated in shaking incubator for 48 hours at 37°C. After incubation time, Nessler's reagent (0.5 mL) was added. Color change was observed (Alhaddad *et al*., 2024).

HCN production assay: TSA medium was prepared and mixed with glycine (4.4g/L). After preparing, the media was autoclaved for 20 minutes at 121°C. After being autoclaved media was dispensed into sterilized petri plates and left to solidify. Then bacterial strains were streaked, and petri plates were covered with Whitman no.1 filter paper that was soaked in 0.5% picric acid solution and in 2% Na_2CO_3 solution. Plates were incubated at 37°C for 1 week. HCN production was detected through color change (Ahmad *et al*., 2008).

Antifungal activity: Dual culture assay was used to check antifungal activity of bacterial strains against *Aspergillus niger* and *Rhizoctonia solani.* Dual culture media was prepared by mixing SDA and TSA in 1:1 to allow both bacteria and fungus to grow on the same media. Bacteria were streaked on medium having fungal disc in center at 2cm distance between them, incubated at 37°C for 7 days. Two sides of plates were kept as negative control, where no bacteria were inoculated (Al-Hussini *et al*., 2019).

Extra cellular cell wall degrading enzymes activities

Protease activity: The selected isolates were checked for protease activity. The cultures of fresh bacterial strains were spotted on the respective medium tryptic soya agar media accompanied with 2% w/v skimmed milk powder and the plates were kept in an incubator at 37°C for 24 hours. After that clear zones were observed on plates after incubation of 24 hours which show indicator of positive activity measured by using vernier caliper (Mushtaq *et al*., 2019).

Cellulase activity: Cellulase activity of the bacterial strains were analyzed by following the protocol of Cattelan *et al*. (1999). A 0.2% Carboxy Methyl Cellulose medium was prepared and was first dissolved in hot water and sterilized. After sterilization, the media was poured into plates and strains were inoculated on the petri plates after its solidification. The incubation for 48 hours, the plates were washed with 0.1% of Congo red dye solution and put in shaker for 20-30 minutes. Zones were observed for yellow halos against red background.

Pectinase activity: To check pectinase activity bacteria were grown on a selective media, which is added with Polyglacturonic acid as a substrate source. Bacteria were spot inoculated with help of sterilized loop on petri plates containing media and placed in incubator at 37°C for 24 hours. After that definite incubation period, the zones formation was measured with the help of Vernier caliper (Raju & Divakar, 2013).

Effect of endophytic bacterial strains on *Triticum aestivum:* The effect of endophytic bacteria on *T. aestivum* growth was assessed through seed priming. Seeds were sterilized, inoculated with bacterial isolates, and incubated in (growth chamber) at 25°C for 14 days. Germination and seedling growth parameters were recorded, with comparisons made between treated and control groups as previously studied by Hu *et al*., (2022).

Statistical analysis

We analyzed the data using the means and standard errors (SEM) of three replicates. The results are presented as mean values with their corresponding standard errors.

Results

Isolation and identification of endophytic bacteria: Bacterial endophytes from roots of *Fagonia indica* were identified through 16S rRNA gene sequence analysis. The identified bacterial strains are presented in Table 1 and Fig 1, with their closest-matching strain, accession numbers and similarity index.

Table 1. Identified endophytic bacterial strains from the roots of *Fagonia indica* with similarity index and accession numbers used in this study.

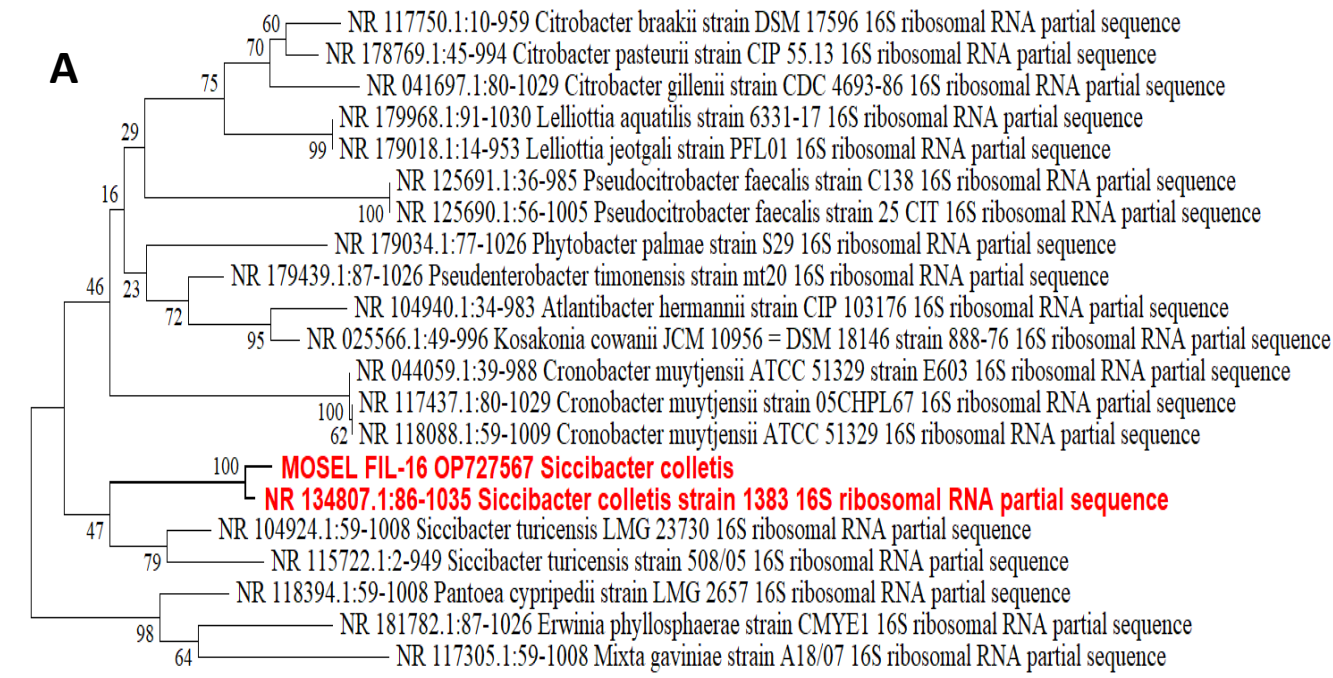
Strain codes	Closest match	Similarity (%) NCBI	Accession No.
MOSEL-FIL 16 (MI-1)	*Siccibacter colletis*	99.79	OP727567
MOSEL-FIL 18 (MI-	*Enterobacter cloacae*	99.57	OP727568

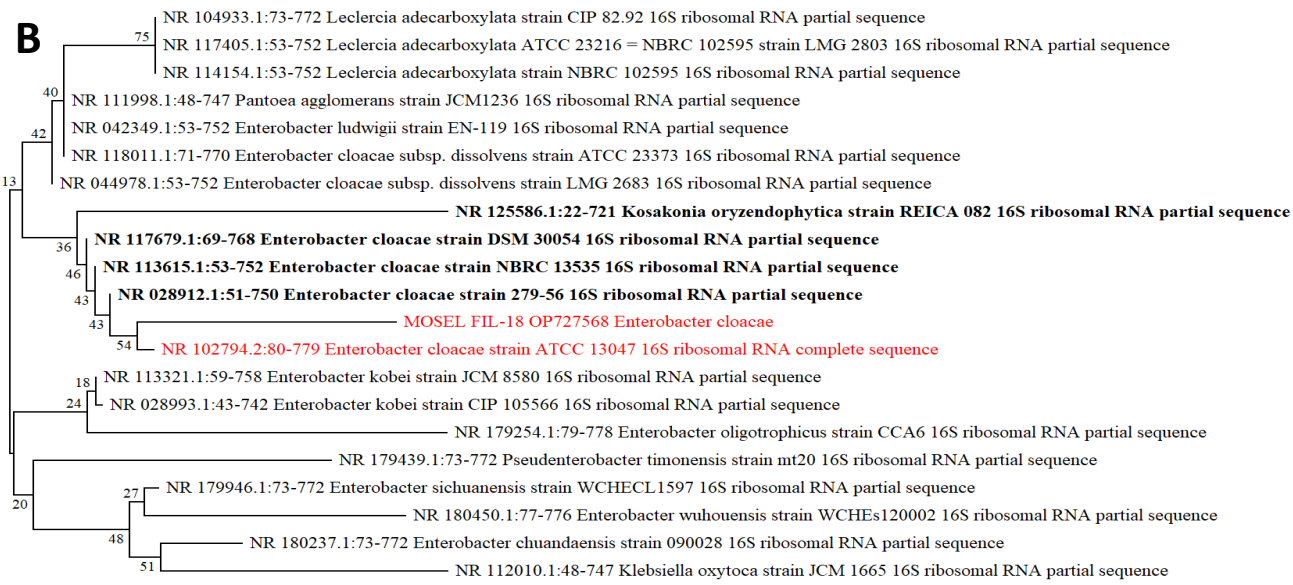
2)				
MOSEL-FIL 20 (MI-3)		*Pseudomonas canadensis*	99.67	OP727569
MOSEL-FIL 24 (MI-4)		*Staphylococcus warneri*	99.86	OP727570

Morphological identification of endophytic bacteria: Bacterial strains were streaked on TSA media at 37°C for 24-48 hours. Colony features i.e., form, texture, elevation, margins, and color of strains were noted (Table 2). Gram's staining confirmed the morphological characteristics.

Biochemical test for identification of endophytic bacteria: Various biochemical tests were performed against selected isolated endophytic bacteria, and the results were recorded after 24 and 48 hours of incubation.

Methyl red test: All the isolates showed positive results, indicating the presence of stable organic acidic end products, as evident by the formation of a distinct red color in the media illustrated in Table 3.





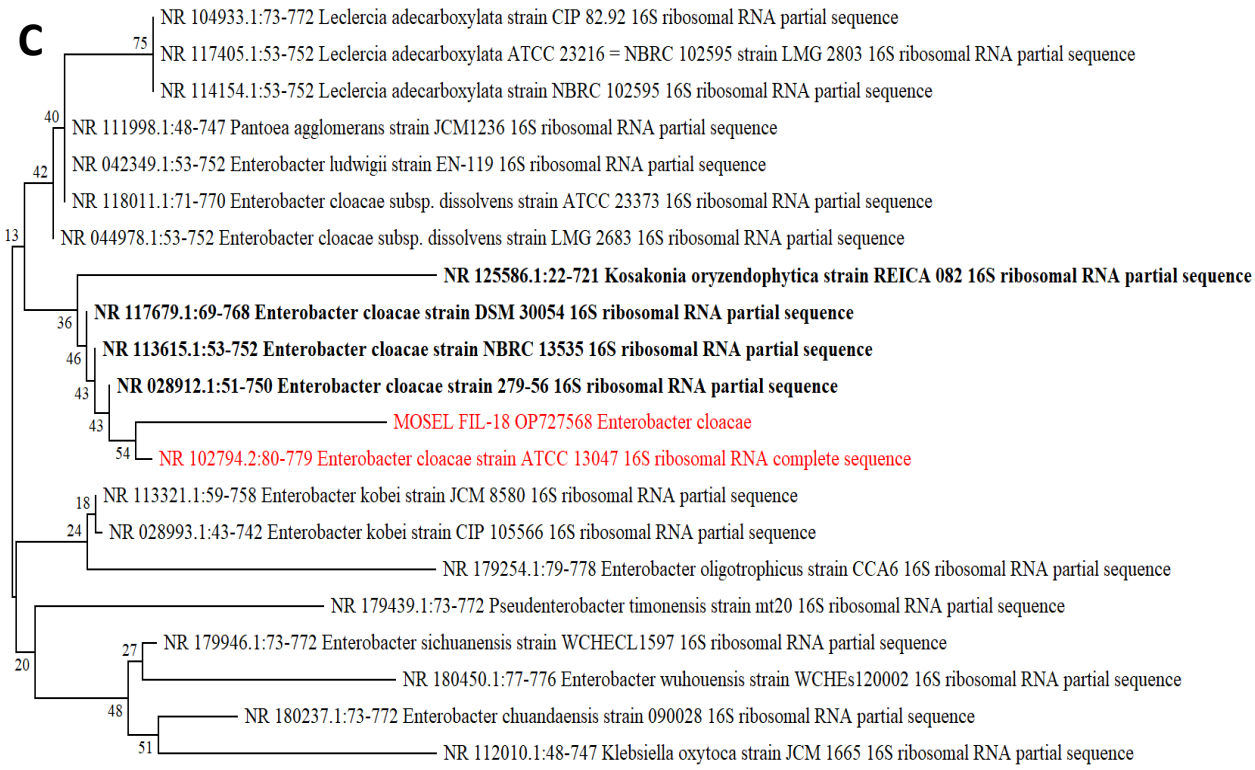


Fig. 1. Phylogenetic analysis of endophytic bacterial isolates A. *Siccibacter colletis* B. *Enterobacter cloacae* C. *Pseudomonas canadensis* D. *Staphylococcus warneri* from NCBI.

Table 2. Morphological identification of endophytic bacterial strains.

Strains	Image	Form	Texture	Elevation	Margins	Color	Gram's staining
MI-1		Irregular	Creamy	Flat	Entire	Light yellow	-
MI-2		Round	Creamy, Sticky	Raised	Undulate	Light yellow	-
MI-3		Round	Slightly creamy	Flat	Entire	Yellow	-
MI-4		Round	Creamy, Sticky	Flat	Entire	White	+

Table 3. Biochemical characterization of bacterial isolates.

Strains ID	Strains name	Methyl red test	Simmon citrate agar test	Mac-conkey agar	Urease test
MI-1	*Siccibacter colletis*	+R	+B	+P	-
MI-2	*Enterobacter cloacae*	+R	-	-	+P
MI-3	*Pseudomonas canadensis*	+R	+B	+P	-
MI-4	*Staphylococcus warneri*	+R	+B	+P	-

+ Indicates growth, - Indicates no growth, B = Blue, P = Pink, R = Red

Table 4. Plant growth-promoting potential of Endophytic bacterial strains assays.

Strains ID	Strains name	Traits of PGPB					Cell wall degrading assays		
		Siderophore production	Phosphate solubilization	Ammonia production	IAA with tryptophan (µg mL^{-1})	IAA without tryptophan (µg mL^{-1})	Pectinase	Cellulase	Protease
MI-1	*Siccibacter colletis*	-	-	+	11.67	8.43	+	-	-
MI-2	*Enterobacter cloacae*	-	+	+	12.31	9.24	+	-	+
MI-3	*Pseudomonas canadensis*	-	+	+	11.14	9.06	+	-	-
MI-4	*Staphylococcus warneri*	+	+	+	19.15	10.68	+	-	+

Positive sign (+) = Presence of positive activity in bacterial isolates, whereas negative sign (–) = Absence of activity

Citrate utilization test: Three isolates MI-1, MI-3 and MI-4 showed the ability to use citrate as a source of carbon by shifting the color of media from green to blue, while MI-2 does not show any color change presented in Table 3.

Mac-Conkey's agar medium test: Three strains MI-1, MI- 2 and MI-3 changed color to pink fermented lactose due to acid production from the lactose while MI-4 showed no growth shown in Table 3.

Urea base agar: MI-2 showed positive results by changing the medium color into pink from yellow due urea hydrolysis while other three strains remained negative results as followed in Table 3.

Biological evaluation of endophytic bacteria for plant growth promotion

Phosphate solubilization: NBRIP medium was used for examining the ability of bacteria to solubilize phosphate. Two strains MI-3 and MI-4 show clear zones after 2 days of incubation, indicating that these two isolates have higher ability to solubilize phosphate. MI-1 and MI- 2 shown activity after 4 days of incubation. Highest solubilization was shown by MI-4 having zone size 2.33mm as mentioned in Table 4.

Siderophores production: CAS agar media was used to check siderophores production by endophytic bacteria. Bacteria showed orange-red halos surrounding their colonies, which shows their capacity of siderophores production. Only MI-4 showed positive results having zone size of 4.45mm in Table 4.

Indole acetic acid (IAA) production: IAA production of all the bacteria was examined in TSB in two conditions; one with tryptophan having concentration 500 µg/mL and other was without tryptophan. The first indication of IAA production by bacteria is appearance of pink color. A small amount of IAA was produced in the absence of tryptophan but more concentration in medium with tryptophan. Maximum IAA amount was produced by MI-4 bacteria in media supplemented with

tryptophan demonstrated in Table 4.

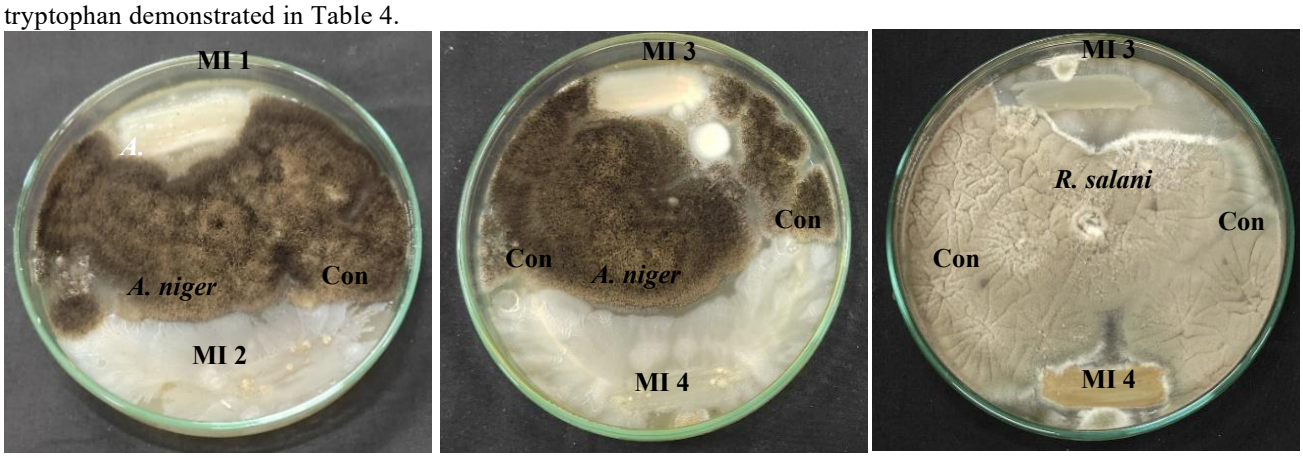


Fig. 2. Antifungal properties of isolated bacterial stains against *Rhizoctonia solani* and *Aspergillus niger*.

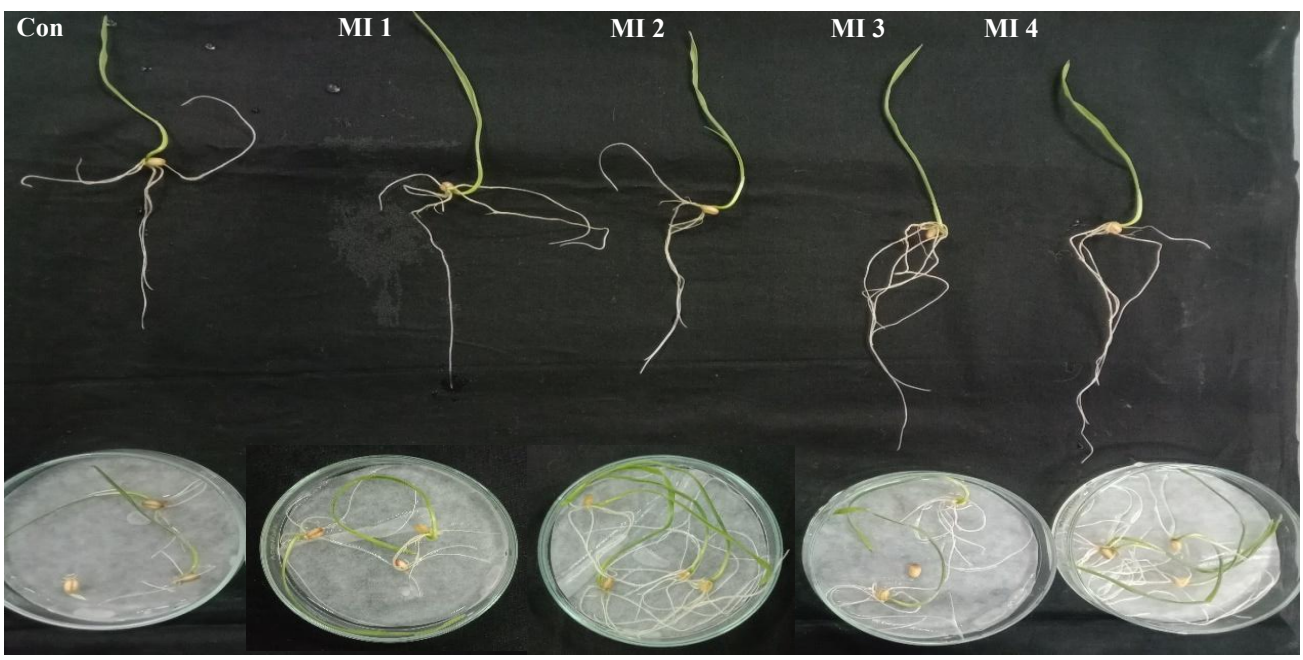


Fig. 4. Pictorial representation of the effect of endophytic bacterial strains on *T. aestivum* growth under *İn vitro* conditions.

Ammonia production: After incubation for 2 days, by the addition of Nessler's reagent into the peptone water, all the strains showed positive results by changing its color in Table 4. The appearance of yellow color indicates ammonia production.

HCN production: Media amended with glycine was used to observe HCN production. All the endophytic bacteria showed positive results, after 4 days of incubation. Positive results were shown by changing the filter paper color from orange to brown. MI-4 showed the highest HCN production by changing filter paper color into dark orange color displayed in Table 4.

Extracellular enzyme activity: Bacterial strains were analyzed for production of various extracellular enzyme activities i.e., pectinase, protease and cellulase. All the bacterial strains showed good results for pectinase activity and formed the zones. Zone size ranged from 1 to 50 mm. Two strains i.e., MI- 2 and MI- 4, show results for protease activity. Zone size ranged from 2m and 9.5mm, respectively in Table 4. None of the strains showed cellulase activity.

Antifungal activity: All the four strains showed antifungal activity, but MI- 2 and MI- 4 showed strongest activity against pathogenic fungus *Aspergillus niger*. Figure 2 signifies three strains MI- 2, MI- 3 and MI- 4 showed antifungal activity, but MI-3 showed highest activity against pathogenic fungus *Rhizoctonia solani*.

Effect of endophytic bacteria on the growth of *Triticum aestivum*: The effect of selected inoculated bacteria on the growth of *T. aestivum* showed that all the selected bacterial isolates significantly enhanced growth under *İn vitro* conditions compared to the control. The bacterial strains *E. cloacae* (MI-2) and *S. warneri* (MI-4) exhibited the maximum growth enhancement by increasing the root length of 60.3 cm and 41.8 cm and shoot length of 14 cm and 10.5 cm respectively as compared to the control. MI-2 and MI-4 not only improved root and shoot lengths but also increased the

fresh weight to 110 mg and 85 mg and dry weight to 18.5 mg and 21.8 mg, respectively, in *T. aestivum* as shown in Figs. 3 and 4.

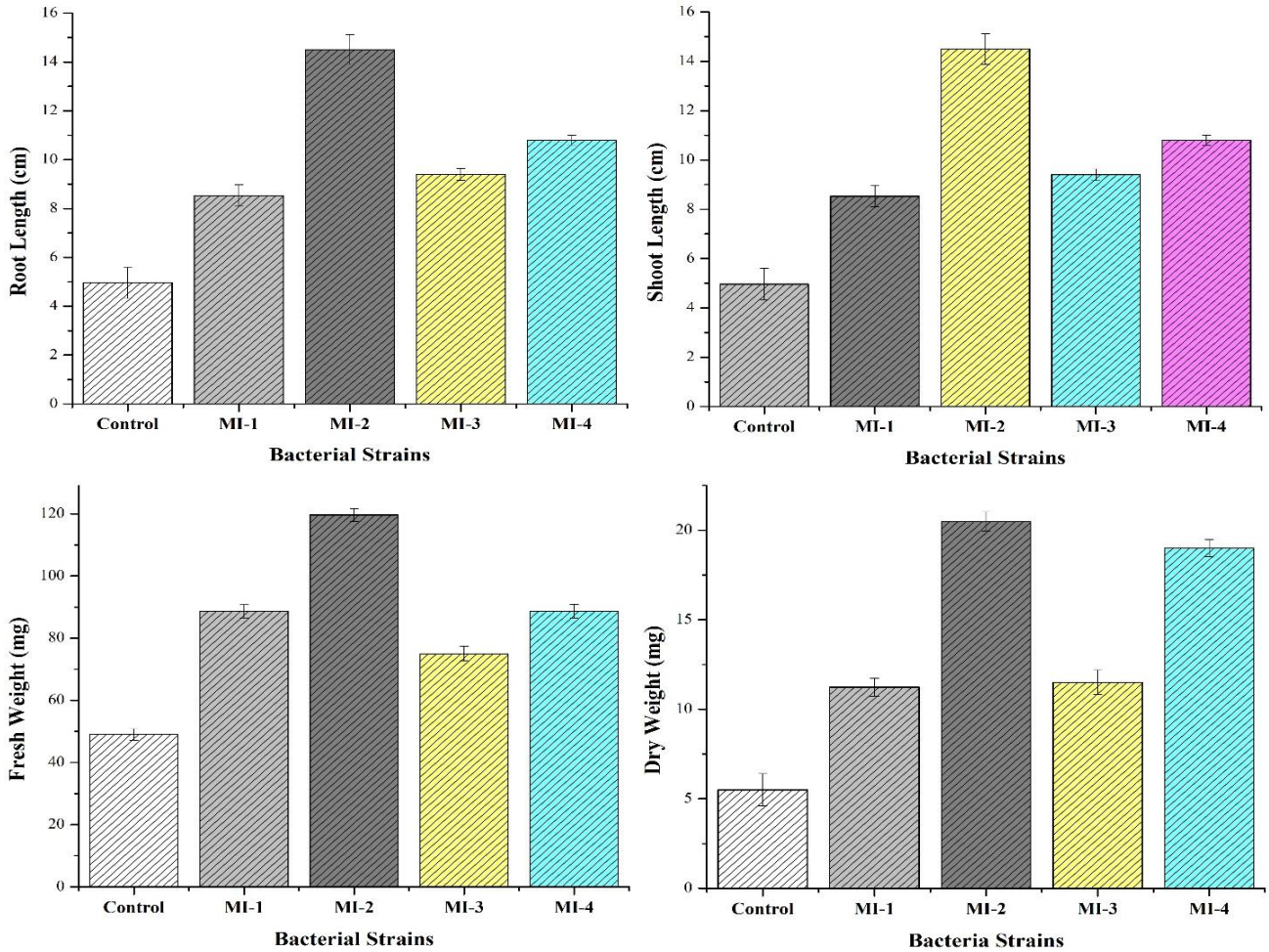


Fig. 3. Impact of endophytic bacterial strains of *Fagonia indica* on Shoot Length of *Triticum aestivum* A. Root length, B. Shoot length, C. Fresh Weight D. Dry Weight. All the values are significantly different.

Discussion

Due to the excessive use of traditional fertilizers, it imposes harmful environmental impacts, which can be reduced by using potassium and phosphorus-solubilizing microbes in farming. This approach promotes sustainable agricultural practices and reduces the dependency on chemical fertilizers (Choi *et al*., 2005). Bacterial endophytes can speed up essential nutrient's availability for plants by solubilizing potassium, phosphorus, and zinc, producing siderophores, and fixing nitrogen. Also, these bacteria shelter the plants from pests and pathogens by the production of enzymes such as xylanases, cellulase, pectinase, gelatinase, and amylase (Rashid *et al*., 2012).

In the current study, the isolated four endophytic bacterial strains from *F. indica* such as *S. colletis*, *E. cloacae*, *P. canadensis*, and *S. warneri* were analyzed through 16S rRNA gene sequence and used to investigate for growth promotion. These isolations confirmed the ability to increase the plant growth through many growth-promoting activities, signifying that they have capability of enhancing the plant growth through multiple mechanisms concurrently. According to Rashid *et al*., (2012), bacterial endophytes must possess a variety of mechanisms to effectively improve plant growth. These mechanisms empower the bacteria to support health and development of plants in multiple ways. In the current experimental work, NBRIP medium was used for examining the ability of bacteria to solubilize phosphate. Two strains MI-3 and MI-4 show clear zones after 2 days of incubation, indicating that these two isolates have higher ability to solubilize phosphate. MI-1 and MI- 2 shown activity after 4 days of incubation. Highest solubilization was shown by MI-4 having zone size 2.2mm. Similar results were found by Azizah *et al*., (2020) who isolates ten PSB isolates were retrieved from maize leaves, stems, and roots, exhibiting high density. Among them, four PSB isolates were recognized as KSB for forming clear zones on Aleksandrov's medium. These isolates exhibited promising potential for phosphate and potassium production, indicating their viability as organic fertilizers to diminish dependence on synthetic ones and enhance macronutrient uptake in maize farming.

This study also proved siderophores production by endophytic bacteria. Bacteria showed orange-red halos surrounding their colonies, which show their capacity for siderophores production. Only MI-4 showed positive

results having zone size of 4.45mm. Misra *et al*., (2024) reported the potential of certain bacteria, like *E. quasihormaechei* (NBRI FY32), known for producing siderophores, in boosting the growth and nutrient uptake of *Spinacia oleracea* under conditions of iron deficiency. Out of 234 isolates examined, NBRI FY32 stood out for its various plant growth-promoting (PGP) characteristics, including its ability to produce ample siderophores, exhibit phytase activity, and degrade oxalate. Inoculation of spinach with NBRI FY32 led to significant enhancements in plant growth parameters and increased nutrient uptake, such as nitrogen, phosphorus, potassium, and iron, both in situations with adequate iron levels and in iron-deficient conditions. This investigation underscores the noteworthy contribution of siderophores-producing endophytic bacteria, exemplified by NBRI FY32, in fortifying spinach plants by elevating nutrient levels and supporting robust plant growth.

For the IAA production, all the bacteria were examined in TSB in two conditions; one with tryptophan having concentration 500 µg/mL and other was without tryptophan. First indication of IAA production by bacteria is appearance of pink color. Calculation of IAA production was done using standard curve and regression equation (R^2=0.993). A small amount of IAA was produced in the absence of tryptophan but more concentration in medium with tryptophan. Maximum IAA amount was produced by MI-4 bacteria in media supplemented with tryptophan. The investigation by Merzaeva & Shirokikh, (2010) also revealed that the Actinomycetes and Coryneform bacteria that have been found on the roots of winter rye were known to produce auxin. In liquid culture, coryneform bacteria generate between 9.0 and 95.0 1g / ml of indolyl-3- acetic acid (IAA), and Actinomycota generate between 39.5 and 83.0 1g / ml of the acid. IAA attains its maximum concentration in Actinomycetes at the point of stationary growth which is under the influence of nutrient medium composition, pH, tryptophan concentration and aeration. Application of these auxins producing bacteria to winter rye seeds increases germination potential and growth of seedlings.

Ammonium productions were checked, all the strains showed positive results by changing their color. The appearance of yellow indicates ammonia production. Afzal *et al*., (2017) investigated isolated endophytic bacteria from *Dodonaea viscosa* belonging to ten different genera that all the strains are involved in production of ammonia, along with showing other plant-growth-promoting activities.

Bacterial strains were analyzed for production of various extracellular enzyme activities i.e., pectinase, protease and cellulase. All the bacterial strains showed good results for pectinase activity and formed the zones. Zone size ranged from 1mm to 50mm. Two strains i.e., MI- 2 and MI-4, show results for protease activity. Zone size ranged from 2mm and 9.5mm, respectively. Sopalun & Iamtham (2020) revealed six bacterial endophytic isolates from medicinal plants, indicating strong antimicrobial activity alongside amylase, protease, and cellulase enzyme production (Vijayalakshmi *et al*., 2016). Furthermore, the 52 strains of endophytes were found in many parts of five epiphytic orchid species from leaf segments. The evaluation of these isolates unveiled their ability to produce diverse extracellular enzymes, particularly lipase, cellulase, and pectinase. Remarkably, the DapR 02 isolated from *Dendrobium aphyllum* roots showed the significant pectinase production, identified as *Pseudopestalotiopsis theae*.

The bacterial inoculum on the growth of *T. aestivum* showed that all the selected bacterial isolates significantly enhanced growth under *In vitro* conditions compared to the control. The bacterial strains *E. cloacae* (MI-2) and *S. warneri* (MI-4) exhibited the maximum growth enhancement by increasing the root length of 60.3 cm and 41.8 cm and shoot length of 14 cm and 10.5 cm respectively as compared to the control. MI-2 and MI-4 not only enhanced root and shoot lengths but also increased the fresh weight to 110 mg and 85 mg and dry weight to 18.5 mg and 21.8 mg, respectively, in *T. aestivum*. Abdel-Hamid *et al*., (2021) supported this idea that bacterial endophytes were applied to *Thymus vulgaris* L. as bio-inoculant. Two endophytic bacterial strains, when applied as a group, they directed to the highest growth performance *of T. vulgari*.

Conclusion

This experiment was conducted aimed to focus on the isolation, molecular identification and evaluation of endophytic bacterial strain from *F. indica* roots to check the potential in promotion of plant growth and antifungal activity. Four bacterial strains (MI-1, MI-2, MI-3, and MI-4) were labeled for analysis based on molecular analysis, morphology and biochemical testing. Morphological colonies revealed variations in color, form, margins, texture, and elevation. Biochemical tests comprising of methyl Red, citrate utilization, Mac-Conkey agar, and urease tests, confirmed a distinctive metabolic capability amongst these strains. Markedly, MI-4 was Gram-positive, while the other three were Gram-negative. Biochemical tests indicated that these bacterial strains possess many plants growth-promoting traits. MI-4 produces siderophores whereas all the strains showed the ability of phosphate solubilization. All strains produced indole acetic acid (IAA) and ammonia, which contributed to increase the plant growth. Additionally, all the strains produced hydrogen cyanide (HCN), and all showed significant antifungal activity, particularly against *R. solani* and *A. niger*. Extracellular enzyme assays revealed pectinase and protease production, with fluctuating efficiency, but cellulase activity was not shown by any strain. The application of bacterial strains on *T. aestivum* improved root and shoot length significantly under *in vitro* conditions, acting as representative potential as biocontrol and biofertilizers agents. Overall, these findings of endophytic bacteria show agriculture sustainability by promoting plant growth and defensive to the plant from pathogens.

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Authors Contribution: Mehwish Iqbal and Tahira Younis conceived the study, designed the methodology, conducted the investigation, performed the experiments, handled software and data visualization, and wrote the original draft of the manuscript. Lubna Rehman helps in isolation and identification of bacterial strains. Nadeem Ullah and Zabta Khan Shinwari contributed to data analysis and manuscript revision.

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