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Research article

Screening and Characterization of Potential Plant Growth-Promoting Endophytes of Wheat (*Triticum aestivum*)

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Abstract

Wheat is the principal and most consumed grain in the world. Biotic factors are known to affect the growth of wheat plants and grain yield worldwide. The aim of the present study was to isolate potential plant growth promoting endophytes. In this study, bacterial endophytes from germinating wheat seeds were isolated, characterized, and screened *in vitro* for PGP traits and then checked for their effects on germination and production of indole acetic acid (IAA), ACC deaminase activity, siderophore production, phosphate solubilization, HCN production, extracellular enzyme production and biocontrol potential. High potential PGPRs were identified by 16 s rRNA sequencing and these strains are *Enterobacter asburiae, Bacillus licheniformis, Achromobacter mucicolens,* and *Pseudomonas fulva*. Antagonistic activity results showed that *B. licheniformis,* and *A. mucicolens* could reduce the growth of the fungal phytopathogens. *Alternaria alternata* and *Fusarium* sp. also produced high levels of indole acetic acid (IAA) with a range of 27.8±0.30 µg/mL, 31.2±0.36, 21±0.20, respectively. Seed germination and development studies showed that superior increase of root and shoot length and weight were observed when compared with uninoculated control plants. The study revealed that the isolated endophytes could be used as plant growth promotion for better plant yield.

1. Introduction

Wheat, *Triticum aestivum* (subsp. *durum*), is a staple food for over 35% of the world's population. It provides more protein and calories than other cultivated crops. Wheat is considered the most nutritious crop in the world [1].

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Wheat (*Triticum aestivum*) is grown in 17% of farmland globally and provides about 82% of the protein source and 80% of the calories for the global population [2-4]. It is grown annually on around 220 million hectares area and approximately 775 metric tons of yield is reported in the world per year [5]. While in India it is grown on around 30 million hectares with 104 metric tons of yield per year, and India is the second leading producer of wheat in the world. To fulfill the need of increasing population, yield has to be increased by 70-104% in 2050 [6, 7].

Wheat production is highly variable due to variations in factors like extreme weather conditions and biological threats which can cause loss of nutritional quality and taste [8, 9]. Additionally, global conflicts like the post pandemic situation after Covid 19 have had a negative impact on wheat production and may pose further threat to wheat markets. Moreover, the public are demanding greener and safer production practices as well as obtaining greater yields by reducing the use of chemical fertilizers. In this situation, alternate approaches are needed in order to develop novel and environmentally friendly technologies such as biofertilizers and bio-stimulants produced from endophytic bacteria, fungi and yeast that have plant growth promotional traits [10-15].

Crop yield and productivity are affected by biotic factors and the uncontrolled use of chemical fertilizers. Biotic factors directly affect plant growth and development by reducing nutritional content and plant strength. Biotic stress is a major contribution to pre as well as postharvest losses [16]. To gain optimum yield, farmers use chemical fertilizers in an uncontrolled fashion, disrupting the environmental balance, degrading soil fertility, and adversely affecting humans [17].

Bacterial endophytes are the microbes present inside the parts of the plants. Endophytes having PGP traits, enhance the growth and health of plants, provide tolerance against different biotic and abiotic factors, and improve crop productivity [17]. Plant growth promoting endophytes (PGPE) are beneficial kinds of bacteria that grow in symbiotic relationships with host plants, leading to stimulation of the growth of the plants [18]. An early-stage development was observed through direct effects on root and shoot growth after inoculation of certain PGPE [17]. In addition, the PGPE also improved biomass production of the crops. PGPEs are important for crops as they directly enhance their growth. PGPEs enhance plant growth by solubilization of phosphate and zinc, and by the production of growth hormones like IAA, gibberellic acid [16]. They also produce HCN, siderophores and different cell wall degrading enzymes like proteases and chitinases which act as biocontrol agents against phytopathogens [18-23].

The present study was planned with the aim to isolate bacterial endophytes from germinating wheat embryos. Endophytes were characterized and screened for their PGP properties. Based on the PGP results, selected PGPEs were identified by 16s rRNA sequencing. Further, the potential of PGP endophytes was checked by *in vivo* seed germination experiments. In this experiment, primary growth of wheat (*Triticum aestivum*) was investigated. These potential PGP endophytes could be used in growth promotor formulation for crops for sustainable agriculture.

2. Materials and Methods

2.1 Isolation and screening of bacterial endophytes

Seeds of wheat (*Triticum aestivum*), of the durum variety, were collected from the fields of Ghaziabad 28.67^{O}N 77.42^{O} E, Uttar Pradesh, and used for isolation of endophytes. Surface sterilization was performed initially by washing with distilled water, followed by washing with 70% ethanol and finally with 1% $HgCl_2$. The same steps were repeated three times [24]. A solution acquired from the last wash (100 µL) was plated on NA in order to check the efficacy of sterilization and used as a control. Ten sterilized seeds were kept on NA plate and incubated for 48 h at 37^{o}C.

Three replicates were prepared. After incubation, different colonies were obtained. Colonies were selected based on morphology. Selected and pure colonies were streaked on NA agar plates and slants for further use [25, 26].

2.2 Morphological and biochemical characterization of bacterial endophytes

The bacterial endophytes were isolated from the sterilized seeds of *Triticum aestivum* and primary identification was done with morphological and biochemical tests using Bergey's Manual. Eight bacterial strains were isolated and morphological characterization was done by gram staining and biochemical characterization was done by various tests such as IMViC test, survival at high temperature test, carbohydrate test (for fructose, maltose, lactose, mannitol, glycerol, sorbitol, starch, xylose, arabinose, glucose, sucrose, rhamnose, adonitol, raffinose, inositol), survival at high salt concentration test, hydrolysis of starch and casein test, catalase and urease test, motility test, acetate utilization test, and nitrate test [27].

2.3 Characterization of bacterial endophytes for plant growth promotional characteristics

2.3.1 Quantitative analysis of indole acetic acid

Quantitative analysis of IAA was carried out in Luria broth with tryptophan. After inoculation, media were incubated for 48 h at 37°C. After incubation, the media were centrifuged, and supernatants were collected and mixed with ortho-phosphoric acid and Salkowski's reagent [28]. The development of pink color confirms the presence of IAA. Optical density (OD) was measured at 530 nm. The OD of samples was compared with standard graph for IAA. The experiment was performed in triplicate [29].

2.3.2 Gibberellic acid estimation

Gibberellic acid was estimated by the colorimetric method [30]. Bacterial endophytes were inoculated in nutrient broth and incubated for 72 h. After incubation and centrifugation, the broth and supernatant were acidified with HCL (pH 2.5), and gibberellic acid was extracted by adding an equal volume of ethyl acetate. The ethyl acetate layer containing gibberellic acid was collected and evaporated to 5 mL. Gibberellic acid was estimated by the colorimetric method and compared with a standard graph.

2.3.3 Qualitative analysis of phosphate solubilization

The phosphate solubilization potential of the bacterial endophytes was estimated qualitatively by streaking the bacterial strains on NBRIP (National Botanical Research Institute's Phosphate growth) medium [31]. After 6 days of incubation, a clear halo zone around bacterial colonies indicates a positive test [32]. Phosphate solubilization test was calculated from formula:

$$\text{Phosphate Solubilization Index} = \frac{\text{Diameter (bacterial colony+ zone)}}{\text{Diameter of bacterial colony}} \quad [33]$$

2.3.4 Zn solubilization

Isolated bacterial endophytes were assayed for qualitative Zn solubilization [20]. Tris-minimal medium with 0.1% zinc oxide (ZnO) were prepared for the test of efficacy of endophytes to solubilize zinc [34]. After spot inoculation of bacterial endophytes, each plate was incubated in the dark for 7 days at 37°C and a clear halo zone was observed [35, 36].

$$\text{Zinc solubilization assay} = \frac{\text{Diameter of clear zone}}{\text{Diameter of bacterial endophytes} \times 100}$$

2.3.5 Production of ammonia

To check the bacterial endophytes' ability to produce ammonia, peptone broth was prepared, and bacterial endophytes were inoculated. After five days of incubation, the supernatants were collected after centrifugation [37]. A color change to yellow after the addition of Nessler's reagent indicates the presence of ammonia. The amount of ammonia is observed based on the color in each tube. A light yellow color indicates a lower amount of ammonia while a dark brown color shows a higher amount of ammonia [38].

2.3.6 Production of siderophore

Siderophore production was assessed using chrome Azurol S (CAS) dye in a nutrient agar medium [39]. All bacterial endophytes were spotted at the center of each plate and incubated at 37°C for 7 days. An orange zone around the colonies indicates a positive test [40].

2.3.7 ACC deaminase assay

Isolated bacterial endophytes were streaked on plates containing Dworkin Foster (DF) minimal medium supplemented with 1-aminocyclopropane-1-carboxylic acid (ACC) and incubated for 4 days at 37°C [41]. Endophytes hydrolyzing the ACC show the presence of enzyme ACC deaminase.

2.4 Production of different enzymes by bacterial endophytes

The production of enzymes like protease, pectinase, chitinase and amylase, and cellulase by bacterial endophytes was assessed [42]. For the detection of protease activity and amylase activity, skim milk agar plates and starch agar plates were streaked with bacterial endophytes, respectively [41]. The plates were incubated at 37°C for 48 h. Clear zones around the bacterial colonies indicate that endophytes can produce the enzymes [43, 44]. Minimal media with 1% colloidal chitin was used for the determination of chitinase enzyme activity. A clear zone around the bacterial colonies confirms chitinase activity [45]. For analysis of the pectinase and cellulase activity of the endophytes, 1% pectin and 1% cellulose were added to the basal medium [46]. Endophytes were streaked on the media and incubated for 48 h. Gram's iodine solution was flooded onto the pectin agar and a clear against the dark-blue background indicates a positive test. In the cellulose medium 0.01%, Congo red solution was added for 15 min and de-stained with 1% sodium chloride solution for 5 min under shaking. A clear zone against the deep-red background shows a positive for cellulase activity [47].

2.5 Biocontrol potential of bacterial endophytes

2.5.1 Fluorescent pigment production

King's B agar media was prepared, and bacterial endophytes were streaked and incubated for 48 h at 37°C. To analyze fluorescent ability, plates were visualized under UV. A slight yellowish green color observed under UV confirms a positive result for fluorescent pigment production [48].

2.5.2 HCN production

Bacterial endophytes were streaked onto King's B medium which had been added with 0.4% glycine, and the plates were covered with filter paper soaked in picric acid in Na_2CO_3 (5%) [49]. After incubation, a change in color from yellow to light brown indicates the production of HCN. Color intensity depends on the amount of HCN produced [50, 51].

2.5.3 Antagonistic activity of bacterial endophytes

Two phytopathogens were selected for the assay of the antagonistic activity of endophytes. *Alternaria alternata* and *Fusarium* sp. were grown on potato dextrose agar plates. Using a cork borer, a disc was removed from each fungus plate and placed in the center of a fresh PDA plate. Bacterial endophytes were then streaked parallelly on both sides of the fungal disc [52]. The plates were incubated for 5 days at 30°C. Inhibition of fungus by bacterial endophytes was observed after incubation and all the plates were compared with control [53].

$$\text{Percent of growth inihibition (\%)} = \frac{\text{Fungal Growth in control} - \text{fungal growth in test plates}}{\text{Fungal Growth in control}} \times 100$$

2.6 Identification of Isolatedendophytes using 16S rRNA sequencing

After preliminary identification and PGPR potential tests of the isolated endophytes, four bacterial strains were screened on the basis of PGPR potential test results, and these strains were subjected to molecular identification by 16s rRNA (1500bp) gene sequencing.

Based on the results of the PGPR potential tests of eight isolated endophytes, out of eight endophytes strains, four strains (S2, S4, S5 and S8) were identified by 16 s rRNA partial sequencing [54].

In this experiment, chromosomal DNA was extracted with a spin column kit (HiMedia). The bacterial 16S rRNA gene (1500 bp) [55] was amplified using polymerase chain reaction in a thermal cycler and was purified using Exonuclease I -Shrimp Alkaline Phosphatase (Exo-SAP) [56]. Purified amplicons were sequenced by the Sanger method in ABI 3500xL genetic analyzer (Life Technologies, USA). Sequencing files (.ab1) were edited using CHROMASLITE (version 1.5) and further analyzed by Basic Local Alignment Search Tool (BLAST). The closest culture sequence was retrieved from the National Centre for Biotechnology Information (NCBI) database to find regions of local similarity between sequences [57]. Nucleotide or protein sequences to sequence in the database is compared and the statistical significance of matches is calculated [58]. The BLAST algorithms are used to infer functional and evolutionary relationships between sequences as well as to help identify members of gene families. An initial search is conducted to find potentially closely related type strain sequences using the BLASTN program [59]. Then, pair wise alignment is done to calculate the sequence similarity values between the query sequence and the sequences identified in the initial search [60].

Therefore, each isolate is reported with the first five-ten hits observed in the said database [61-63]. Further phylogenetic analysis for species prediction and evolutionary relationship was performed using MEGA 11.

2.7 Seed germination study

This experiment was designed to check the effect and potential role of isolated bacterial endophytes in growth promotion. Wheat (*Triticum aestivum*) seeds were selected for the study of the early-stage development of wheat [53]. Bacterial endophytes were grown in NB medium and after 24 h of incubation, OD measurements were taken. When the ODs of all the strains had reached 0.5, the sterilized wheat seeds were treated with respective bacterial endophytes, and the control seed were treated with uninoculated media. Five seeds were sown in each plastic cup with autoclaved soil and all experiments were performed in triplicate. The cups were kept in a growth chamber for 21 days and irrigated and observed daily. After germination of seeds, the growth parameters were studied [64, 65].

2.8 Statistical analysis

All the data presented here were statistically analyzed. Values presented in Tables are mean±SD, and data obtained from the seed germination study was analyzed by ANOVA. Comparative analysis was done by the LSD test on all the means from the data set at a significance level of ≤ 0.05 [66].

3. Results and Discussion

3.1 Isolation of bacterial endophytes from germinating wheat

A total of eight endophytes (S1, S2, S3, S4, S5, S6, S7, and S8) from the germinating wheat seeds were isolated and streaked on NA medium, incubated at 37°C, and then stored at 4°C for further studies.

3.2 Morphological characterization of bacterial endophytes

Based on the gram staining results, it was found that the isolated bacterial endophytes were gram-negative and gram-positive. The gram staining results of the bacterial endophytes are shown in Table 1.

Table 1. Gram staining results

Strain Name	Shape	Gram Reaction
S1	Rod shape	Gram+ve
S2	Rod shape	Gram-ve
S3	Rod shape	Gram-ve
S4	Rod shape	Gram+ve
S5	Long rods	Gram-ve
S6	Rod shape	Gram+ve
S7	Short rods	Gram+ve
S8	Rod shape	Gram-ve

3.3 Biochemical characterization of bacterial endophytes

The isolated bacterial endophytes were identified by different biochemical tests, and the results obtained are shown in Table 2.

Table 2. Biochemical tests for the isolated endophytes

Biochemical Test		Isolated Strains							
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
Carbohydrate Test	Fructose	-	+	-	-	+	-	+	+
	Maltose	-	+	-	-	+	-	+	+
	Lactose	+	+	-	+	-	+	+	+
	Mannitol	+	+	-	-	-	+	+	+
	Glycerol	-	+	-	-	+	-	+	+
	Sorbitol	-	-	-	-	-	-	+	-
	Starch	+	+	-	-	+	-	+	+
	Xylose	-	+	-	-	+	-	+	+
	Arabinose	+	+	-	+	-	-	-	-
	Glucose	-	+	-	+	+	+	+	+
	Sucrose	+	+	-	-	-	-	+	+
	Rhamnose	+	+	-	-	+	-	+	+
	Adonitol	-	+	-	+	+	+	+	+
	Raffinose	-	+	+	-	+	-	-	+
	Inositol	+	-	-	-	+	-	-	-
IMViC	Indole	-	-	+	-	+	-	-	+
	MR	-	+	-	-	-	+	+	-
	Citrate Agar	-	-	-	-	-	-	+	-
	VP	-	-	-	+	+	-	-	-
High Salt	7 % NaCl	-	+	+	+	+	+	+	+
High Temperature	Growth at 45°C	+	+	+	+	-	-	+	+
	Catalase	-	+	+	+	+	+	+	+
	Casein	-	-	+	+	+	-	+	+
	Urease	-	+	+	+	-	+	-	+
	Starch Hydrolysis	-	+	+	+	-	+	-	-
	Nitrate	-	-	+	-	+	+	+	-
	Acetate	-	-	+	-	-	-	-	+

3.4 Characterization of endophytes for plant growth promotional (PGP) traits

All eight strains were analyzed for PGP potential, quantitative analysis of indole acetic acid, gibberellic acid content, qualitative analysis of phosphate solubilization, production of ammonia and siderophores, and ACC deaminase assay, and the results are shown in Table 3. All the bacterial endophytes were positive for indole acetic acid production except for strain S1, S3 and S7. *Bacillus licheniformis* S4 and *A. mucicolens* S5 produced 27.8 and 31.2 µg/mL of IAA, respectively. All bacterial endophytes showed a positive result for phosphate solubilization, except for strain S7. Five bacterial endophytes produced the ACC deaminase enzyme, and it was concluded that endophytes had the potential to abate the ethylene level in crops. All the strains except for S1 and S6 produced siderophores, a result that was confirmed by CAS assay. Strains S2, S4, S5 and S8 were capable of solubilizing zinc.

The ability of endophytes to enhance seed germination and elongation of shoots was assessed via gibberellic acid (GA_3) production assay, which revealed that *A. mucicolens* S5 produced gave the highest amount of GA_3. The endophyte production of ammonia was assessed quantitatively. it is another important indicator of the potential to stimulate the plant growth of bacterial endophytes (Table 3).

Table 3. Characterization of PGPR activities of endophytes isolated from wheat genotype

Isolated Endophytes	PGPR Activities						
	IAA (µg/mL)	Phosphate Solubilization	GA_3 Production (µg/mL)	Ammonia	ACC Deaminase Activity	Siderophore	Zn Solubilization
S1	-	10.6±0.43	-	-	+	-	-
S2	18.03±0.15*	13.1±0.1	23.26±0.47	+	+	+	11.1±0.1
S3	-	8.3±0.26	-	+	_	+	-
S4	27.8±0.30	14.33±0.32	27.03±0.66	+	+	+	20.53±0.81
S5	31.2±0.36	15.06±0.20	32.73±0.56	+	+	+	13.46±0.37
S6	15.06±0.20	7.66±0.32	12.16±0.30	-	+	-	-
S7	-	-	-	+	-	+	9.3±0.26
S8	21±0.20	11.56±0.35	12.06±0.25	+	+	+	15.03±0.15

*Values are mean ± standard deviation of three independent experiments (n=3)

3.5 Production of different enzymes by bacterial endophytes

The production of enzymes like protease, cellulase, chitinase, and amylase, by the bacterial endophytes was assessed. Strains S2, S3, S4, S5, S7 and S8 were found to be producers of all the enzymes tested. In the protease test, a clear zone was observed in all the strains except for S1, and the largest zone was observed for *E. asburiae* S2. (Table 4).

3.5.1 Biocontrol potential of isolated endophytes

The biocontrol potential of endophytes was tested by qualitative estimation of fluorescent pigments and via the HCN test, and the results are shown in Table 5. All the strains except for S3 and S7 were potential agents for fluorescent pigment production, As shown by the HCN production test, all the strains were capable of hydrogen cyanide production except for strains S1 and S6.

Table 4. Results showing cell wall degrading enzymes production by isolated endophytes

Isolated Endophytes	Cell Wall Degrading Enzyme Production by Endophytic Bacterial Isolates			
	Protease	Cellulase	Chitinase	Amylase
S1	-	-	-	-
S2	20±0.9*	12.1±0.1	14±0.1	18.27±0.45
S3	10.7±0.52	10.36±0.55	13.13±0.15	14.64±0.30
S4	17.5±0.55	13.96±0.05	16.26±0.20	16.33±0.49
S5	18.2±0.97	15±0.1	15.33±0.25	16.83±0.72
S6	11.8±1.57	14.53±0.30	-	11.83±0.70
S7	12.6±1.37	-	-	10.7±0.43

*Values are mean ± standard deviation of three independent experiments (n=3).

Table 5. Results showing biocontrol potential by fluorescent pigment and HCN tests

Isolated Endophytes	Fluorescent Pigment	HCN
S1	+	-
S2	+	+
S3	-	+
S4	+	+
S5	+	+
S6	-	-
S7	+	+
S8	-	+

3.5.2 Antagonistic activity of endophytes

Another test of the biocontrol potential of the endophytes was done by *in-vitro* antagonistic test. Two phytopathogens, *Al. alternata* and *Fusarium* sp., were selected for antagonistic activity of bacterial endophytes. Inhibition was observed after incubation and all the plates were compared with control. Four bacterial endophytes, *Enterobacter asburiae, Bacillus Licheniformis* (NR118996), *Achromobacter mucicolens* and *Pseudomonas fulva*, showed good percentage of inhibition against both the phytopathogens tested (Table 6). These bacterial endophytes are potential agents to be used to control disease-causing phytopathogens.

3.6 Identification and phylogenetic analysis of the bacterial endophytes by 16S rRNA sequencing

Based on the result of PGPR activity of all eight isolated endophytes, four strains showed higher activity in all the PGPR tests. These four strains, S2, S4, S5 and S8, were selected for further study, and sent for the sequence analysis of 16S rRNA and NCBI BLAST was done to check the phylogenetic relations of the isolated endophytes, and phylogenetic trees were constructed using MEGA 11. Our endophytic isolates belonged to four different genera, namely *Enterobacter, Bacillus, Achromobacter* and *Pseudomonas.* S2 showed high identity (94%) with *Enterobacter*

asburiae (NR024640), S4 showed 99.74% similarity with *B. licheniformis* (NR118996) while S5 matched 99.22 % identity to *Achromobacter mucicolens (*NR117613). S8 showed 94% similarity with *Pseudomonas. fulva (*NR104280). All the sequences obtained after sequencing were deposited to GenBank and the accession numbers were obtained (Table 7, Figure 1).

Table 6. Antagonism by isolated strains S1 to S8 against fungal phytopathogens, *Altenaria alternate* and *Fusarium* sp.

Strain Name	Percentage of Relative Inhibition Against Fungal Phytopathogens (Zone Diameter in mm)	
	Alternaria alternata	***Fusarium* sp.**
S1	ND	47.73±1.28
S2	64.02±1.51*	74.35±1.28
S3	36.40±3.31	48.28±1.60
S4	73.68±1.32	69.22±1.04
S5	68.41±1.31	77.34±1.95
S6	48.68±3.95	ND**
S7	47.80±2.74	39.31±1.96
S8	60.08±2.01	72.21±1.47

*Values are mean ± standard deviation of three independent experiments (n=3).

Table 7. Similarity indexes of isolated endophytes based on 16s rRNA sequencing results

Strains	Accession No of 16s rRNA Sequences	Best Closest Match	Accession No. NCBI (GenBank)	Similarity (%)
S2	NR_024640	*Enterobacter asburiae*	OL_966967	(94%)
S4	NR_118996	*Bacillus licheniformis*	OL_966968	(99.74%)
S5	NR_117613	*Achromobacter mucicolens*	OL_966969	(99.22%)
S8	NR_104280	*Pseudomonas fulva*	OL_966970	100%

3.7 Seed germination study

After 21 days of germination, all the treatment sets were observed and analyzed statistically. All treatments with bacterial culture were compared with the uninoculated control and all the parameters of plant growth were studied (Figure 2, Tables 8 and 9 and Figures 3-5).

We found that the experimental setup treated (inoculated) with bacterial culture showed significantly higher growth as compared with the control. The growth of both shoots and root was found to be higher than the control (approx. 6-10% increase is seen in inoculated samples). We also measured the dry and wet weight of shoots and roots. Based on the result, we concluded that the bacterial endophytes have potential for plant growth promotion.

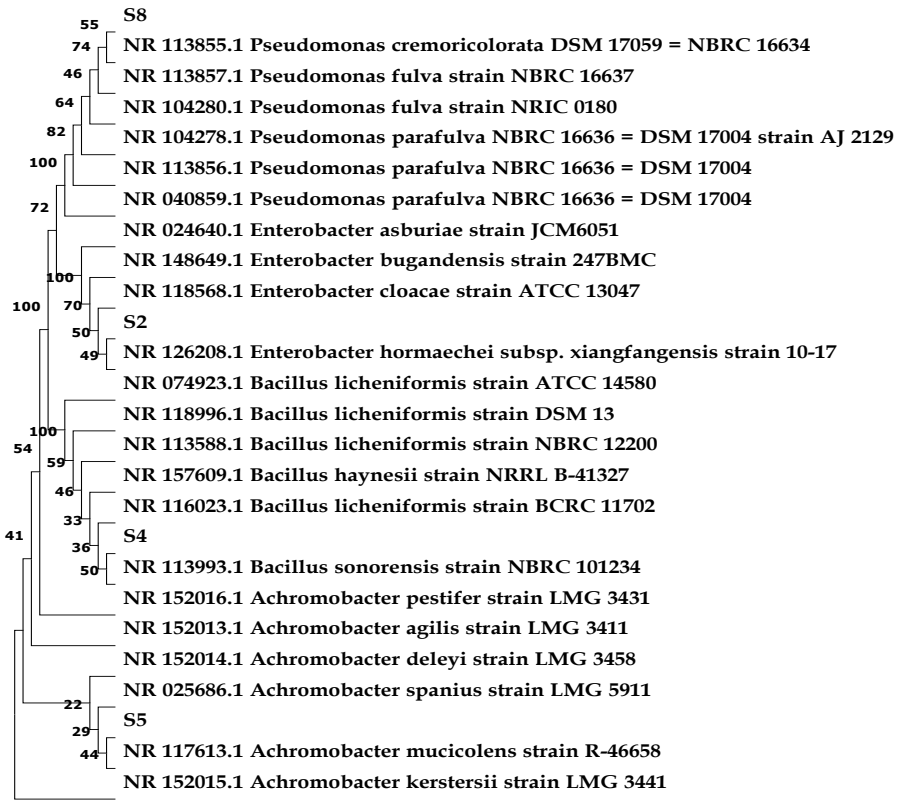

Figure 1. Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of the wheat endophytes S2, S4, S5 and S8 and some of their closest phylogenetic taxa

Figure 2. Seed germination experiments showing the effect of isolated endophytes on growth performance of *Triticum aestivum* (A) Control; (B) *T. aestivum* seeds inoculated with *Enterobacter asburiae* S2; (C) *T. aestivum* seeds inoculated with *Bacillus licheniformis* S3; (D) *T. aestivum* seeds inoculated with *Achromobacter mucicolens* S4; (E) *T. aestivum* seeds inoculated with *Pseudomonas fulva* S5.

Table 8. Effect of bacterial endophytes on the root and shoot length of *Triticum aestivum*

	Root Length (cm)	Shoot Length (cm)	Germination Rate (%)	Total Height (cm)	Vigor Index VI=RL+SL×GP
Control**	1.96±0.04^{b}*	7.20±0.60^{c}	100	9.19	919
S2	2.10±0.45^{b}	8.06±0.60^{bc}	100	10.16	1016
S4	2.86±0.41^{a}	7.93±0.41^{c}	100	10.8	1080
S5	2.24±0.30^{ab}	9.26±0.32^{a}	100	11.5	1150
S8	1.80±0.45^{b}	8.86±0.35^{ab}	80	10.66	1066

*Values within the same column with different letters are significantly different by ANOVA and LSD test ($p \leqslant 0.05$), values are means±SD. **Control is uninoculated plant (n=3).

Table 9. Effect of bacterial endophytes on root and shoot weight of *Triticum aestivum*

	Fresh Weight (mg)		Dry Weight (mg)	
	Shoot Weight	Root Weight	Shoot Weight	Root Weight
Control**	203.81±4.36^{e}*	460.60±5.36^{d}	90.0±3.29^{d}	159.60±2.84^{d}
S2	277.03±4.90^{d}	541.10±4.27^{c}	98.4±2.54^{c}	180.36±2.80^{c}
S4	319.90±4.35^{c}	577.60±4.30^{b}	101.6±2.47^{c}	194.60±3.05^{b}
S5	378.06±5.44^{a}	601.40±5.36^{a}	122.3±2.81^{a}	210.60±4.03^{a}
S8	344.36±5.26^{b}	606.30±4.31^{a}	116.0±1.95^{b}	199.06±4.50^{b}

*Values present in the same column with different letters are significantly different by ANOVA and LSD test ($p \leqslant 0.05$), values are means±SD. **Control is uninoculated plants (n=3).

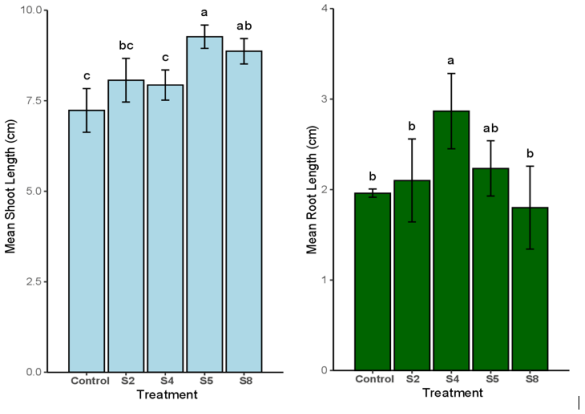

Figure 3. Effect of bacterial endophytes on root and shoot length of *Triticum aestivum*
Error bars are shown in the graph means±SD. Bars with the same letter for each bacterial endophyte are not significantly different and different letters on bars indicate that mean values are significantly different. ($P \leq 0.05$).

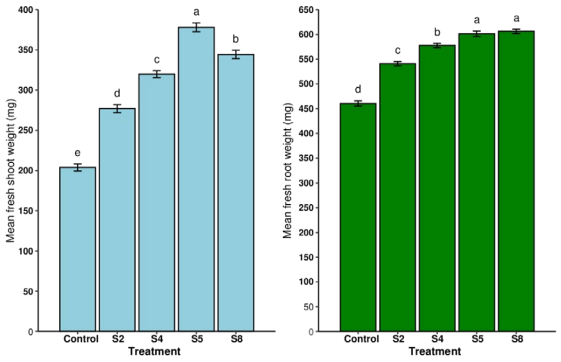

Figure 4. Effect of bacterial endophytes on shoot and root weight (fresh) of *Triticum aestivum* Error bars are shown in the graph means±SD. Bars with the same letter for each bacterial endophyte are not significantly different and different letters on bars indicate that mean values are significantly different ($P \leq 0.05$).

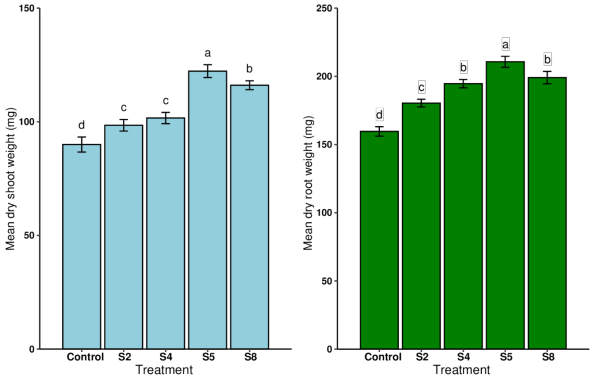

Figure 5. Effect of bacterial endophytes on root and shoot weight (dry) of *Triticum aestivum* Error bars are shown in the graph means±SD. Bars with the same letter for each bacterial endophyte are not significantly different and different letters on bars indicate that mean values are significantly different ($P \leq 0.05$).

In this study, eight putative bacterial endophytic strains were isolated from wheat (*T. aestivum*) seeds of the durum variety. Eight bacterial endophytes were isolated and identified by different biochemical tests and 16S sequencing. The plant growth promoting properties of these bacterial strains were characterized by measurement of extracellular enzyme production, IAA, GA_3 production, HCN production siderophore, ACC deaminase, ammonia production, fluorescent pigment, and phosphate and zinc solubilization. Based on the PGP characteristics, four endophytic bacterial strains were selected to evaluate their effects on plant growth and development

The bacterial endophytes exhibited different enzymatic activities involving cellulase, protease, amylase, and chitinase production. Cellulolytic and proteolytic activities are known to enable microorganisms to penetrate plant tissues and establish a symbiotic relationship with their host plants. The isolate strains such as *Enterobacter asburiae* strain S2 showed high proteolytic activity and *Ps. fulva* strain S8 showed high hydrolytic activity for cellulose, chitin, and amylase. Similarly, endophytic *Ps. fulva* S8 was shown to be a good producer of cellulase, chitinase, and

amylase. The extracellular hydrolytic enzymes produced by endophytes contribute indirectly to plant growth promotion and protection against pathogens [38]. The endophytes can be described as bio producers for amylases and xylanases based on their amylolytic and xylanolytic activities. Moreover, these enzymes enable endophytes to penetrate plant tissues and build a symbiotic relationship with their host plant, which is indicative of their potential use in agricultural and industrial applications [44, 67].

Endophytic bacteria can indirectly assist plant growth through the production of substances that inhibit plant pathogens [50, 51]. Our results also confirm this, and we report that four endophytic bacterial strains exhibited good antagonistic effects against two plant pathogens, *Al. alternata* and *Fusarium* spp. Out of four strains, *E. asburiae* strain S2 showed 89.82% inhibition against *Rhizopus oryzae* while *B. licheniformis* strain S4 showed 79.66% inhibition against *Aspergillus ochraceus*. About 75% of isolated endophytes were able to produce HCN and fluorescent pigments, which can inhibit the development of plant diseases. The study was also focused on studying their role in plant growth promotional activity. Such activities include solubilization of insoluble tricalcium phosphate, ACC deaminase activity, and siderophore production.

The PGP properties of bacteria like ammonia and IAA production, as well as P-solubilization, are among various mechanisms exhibited by bacteria that enhance plant growth. In the present study, most of the endophytic bacterial isolates were able to produce different amounts of ammonia. So these bacteria can supply ammonia as a nitrogen source for plant growth. These endophytes can enhance plant growth through the production of ammonia by the hydrolysis of urea into ammonia and carbon dioxide [64]. With regard to P-solubilization, about 87% of the isolated endophytes showed variable capacity to solubilize phosphate. Zinc is also a very important micronutrient for plant growth, The isolated endophytes were able to solubilize zinc leading to solubilizing complex forms of zinc into simple forms. Inoculation of these P-solubilizing endophytic bacteria leads to an increase in pant growth performance.

All the endophytic bacterial strains had the ability to produce high IAA concentrations in range of 15.06 to 31.2 µg/mL. Thus, in the current study, a high capacity to produce IAA was selected to determine their effect on wheat growth performance. The results showed that *B. licheniformis, A. mucicolens* could reduce the growth of phytopathogens with 40-70% inhibition and could also produce high indole acetic acid (IAA) content in the ranges of 27.8±0.30 µg/mL, 31.2±0.36 µg/mL, and 21±0.20 µg/mL, respectively. GA_3 in the ranges of 27.03±0.66 µg/mL and 32.73±0.56 µg/mL was produced by *B. licheniformis* S4 and *A. mucicolens* S5, respectively. The effect of inoculants on crop yields was previously studied in pot experiments by a few researchers. Our investigation on the effect of inoculants on germination and growth focused on measurement of the effect of isolated endophytes individually on wheat plants. The increase in the shoot and root length and weight (fresh and dry), and in the vigor index (approx. 6-10% gain) was observed for these parameters compared to the non-inoculated control and was probably due to release of metabolites by bacteria and mineralization of nutrients which made them easily available for plants [36, 54].

4. Conclusions

The present study showed that bacterial endophytes isolated from *T. aestivum* seeds had PGP properties. Selected endophytes showing good PGPR properties belonged to four different genera, namely *Enterobacter, Bacillus, Achromobacter* and *Pseudomonas*.The dual roles of PGPR along with antagonistic activity of *E. asburiae* S2, *B. licheniformis* S4, *A. mucicolens* S5, and *Ps. fulva* S8 made them appealing alternatives to hazardous fumigants and fungicides. On the basis of PGP

potential and seed germination results, *A. mucicolens* S5 and *Ps. fulva* S8 can be very good potential bioinoculants. This study provides a benchmark for the potential role of these PGP endophytes that are able to enhance plant growth, and have the capability to control phytopathogens. The identified endophytes can be used as active biological agents for agriculture sustainability. This study provides evidence of the potential of these endophytes to improve plant production and plant health, leading to improved soil quality and fertility in the agricultural sector. Further investigation of their mechanisms of colonization and competition against other soil microbial communities and their efficacy at a field level will be required.

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