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# PLANT GROWTH PROMOTING POTENTIAL OF ENDOPHYTIC BACTERIA ISOLATED FROM THE ROOTS OF *BERBERIS LYCIUM* ROYLE.

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**Abstract**

*Berberis lycium*, a wild shrub, is a therapeutic plant that can tolerate harsh environmental conditions. Endophytic bacteria associated with it act as a repository for therapeutic compounds. It contributes significantly to the production of a diverse array of bioactive compounds. The aim of the current study was to isolate and identify endophytic bacteria from the roots of *Berberis lyceum* and screen them for both plant growth promoting traits and bioactive metabolites. Five bacterial strains belonging to the genus *Bacillus* were chosen and identified. These bacteria exhibited phosphate solubilization, ammonia production, IAA production and protease activity. *Bacillus subtilis* showed substantial root elongation in canola, potentially attributable to its favorable plant growth-promoting (PGP) traits. Variation was observed in the total flavonoid and phenolic contents among the bacterial extracts. *Bacillus paramycoides* showed significantly high phenolic content (183.1µg QA/mg), *Bacillus subtilis* showed significantly high flavonoid content (58.5µg GAE/mg), reducing power (RP) 196 µg/mg of crude extract, total antioxidant capacity (TAC) 170.1 µg/mg of crude extract, and DPPH activity demonstrated an IC50 value of 36.8 µg/mL. GC-MS analysis of crude extract from *Bacillus subtilis* confirming the presence of fatty acids with chain lengths ranging from $C_8$ to $C_{24}$ suggests the diverse composition of lipids. Our findings revealed isolated strains and their extracts possess plant growth promoting traits and bioactive compounds that highlight them as a promising and abundant source of metabolites. The application of these metabolites could potentially reduce the reliance on agrochemicals in food and drug production.

**Key words:** *Bacillus*, Endophytic bacteria, *Berberis lycium*, Phenolic compounds, Antioxidant activity.

## Introduction

Bacteria commonly inhabit both the surface and internal tissues of most plants. An endophyte refers to a bacteria or fungi that lives inside plant tissues without causing any apparent harm to the plant. Endophytes establish themselves within the internal parts of host plants and can engage in various relationships such as symbiotic, mutualistic, or trophobiotic interactions (Adeleke *et al*., 2021). There has indeed been a growing interest in endophytic bacteria in recent years, particularly due to their potential benefits for plants. Some of these bacteria are recognized for their ability to enhance nutrient availability, produce growth hormones, confer stress tolerance, stimulate systemic resistance, or ward off plant pathogens. Plants infected with endophytes frequently exhibit accelerated growth compared to non-infected plants, partially attributable to the production of phytohormones by these endophytes (Mohamed *et al*., 2024). Endophytic bacteria, which reside in various healthy plant parts such as fruits, vegetables, stems, and roots, enter primarily through the root zone. However, they may also utilize flowers, stems, cotyledons, or germinating radicles as entry points. Once inside a plant, endophytes may remain localized at the entry point or disseminate throughout the plant's tissues (Boukhatem *et al*., 2022).

The exploration of endophytes presents a promising avenue for agricultural research. Endophytic bacteria in wild and medicinal plants not only contributes to our understanding of plant-microbe interactions in natural ecosystems but also holds significant potential for applications in agriculture. Identifying and harnessing the beneficial endophytic bacteria could lead to the development of novel biotechnological solutions for improving crop productivity, resilience, and sustainability (Afzal *et al*., 2017). This approach represents a promising strategy for sustainable agriculture, offering opportunities to reduce reliance on chemical inputs while promoting ecological balance and resilience in farming systems.

*B. lycium*, known as Kashmal in Hindi, and Ishkeen in Urdu, belongs to the Berberidaceae family. Locally named Kawdach in the Kashmir valley, it has been traditionally utilized by tribal communities in Jammu and Kashmir, India, for generations. This evergreen shrub, reaching heights of 2-3 meters, thrives mainly in the Himalayan regions. Its roots, bark, stems, leaves, and fruits are commonly used as both medicine and food. Renowned for its medicinal properties, *B. lycium* has gained widespread acceptance in Ayurvedic medicine. It is acknowledged for its ability to address various health issues such as liver disorders, abdominal ailments, cough, ophthalmic issues, skin conditions, oral ulcers, conjunctivitis, piles, kidney diseases, and leprosy. Pharmacological investigations have revealed its diverse therapeutic effects, including antihyperlipidemic, hypoglycemic, antipyretic, hepatoprotective, antimicrobial, antifungal, anticancer, and pesticidal properties (Parra *et al*., 2018).

Endophytes have gained attention for their crucial roles in enhancing plant growth and survival, especially under adverse conditions (Shen *et al*., 2019). Recent research on endophytes has shifted towards cellular and molecular studies, providing valuable insights into their future commercial development. Metabolomic studies have uncovered endophytes as reservoirs of novel bioactive secondary metabolites (Gouda *et al*., 2016; Yadav, 2018). The emergence of endophytes in microbial biotechnology has opened new avenues in various fields including agriculture, medicine, and industry (Rajamanikyam *et al*., 2017; Gouda *et al*., 2016). Endophytic bacteria and their metabolites offer promising

alternative options such as chemical pesticides that can inhibit the growth of potential plant pathogens and enhance crop productivity. Exploring the diverse roles of endophytes in ecosystems can greatly enhance their applications in agriculture, particularly in plant growth and increasing crop yield (Gao *et al*., 2022). Specifically, studies on endophytes from *B. lycium* concerning plant growth promotion particularly in the context of their bioactive compounds, total phenol compounds, and antioxidant properties are limited. Therefore, this research is centered on the exploration of bioactive compounds, total phenol compounds, and their antioxidant properties derived from endophytic bacteria associated with *B. lycium.* Although some work has been done on therapeutic potential of endophytes isolated from stem and leaves of *B. lycium* (Nisa *et al*., 2022).

## Material and Methods

**Collection of plant material:** Plants samples were collected from Nakyal village, Kotli, Azad Kashmir, Pakistan, were collected in sample bags and brought to the Molecular Systematics and Applied Ethnobotany Lab (MOSAEL) for endophytic bacterial isolation. The plant was taxonomically classified as *B. lycium* by the Department of Botany at Quaid-e-Azam University, Islamabad.

**Isolation of endophytic bacteria:** The roots of the plant were thoroughly cleansed first with tap water and then with double distilled water to eliminate any soil residue. Segments approximately 0.5-1 cm long were dissected from the sample, then undergo surface sterilization in a laminar airflow cabinet, being soaked in 70% ethanol for 2 mins, followed by a 5 min treatment with Clorox (commercial bleach), and then rinsed thoroughly with sterile distilled water. After drying gently on blotting paper, 5–6 root pieces were placed on TSA (Tryptic Soy Agar) medium for isolating endophytic bacteria. The plates were then incubated at 28°C for 24 h to encourage bacterial colony growth (Rehman *et al*., 2017). For further studies, pure and morphologically different colonies were chosen and preserved in glycerol stocks at -80°C .

**Genotyping identification:** The molecular identification of endophytic bacteria was done after the extraction of DNA through the plain boiling method (Yamagishi *et al*., 2016). The 16S rRNA gene was amplified using universal bacterial primers 27F (5′-CAGAGTTTGATCCTGGCT-3′) and 1492R (5′-AGGAGGTGATCCAGCCGCA-3′) in PCR, producing a 1465-base pair product (Chen *et al*., 2010). Subsequently, the purified PCR samples were subjected to commercial Sanger sequencing with the 27F primer at Microgen (South Korea). The obtained sequences were compared with the GenBank database, and the near full-length 16s rRNA gene sequences were deposited in GenBank under accession numbers PP231775-PP231779.

### Screening for growth promoting parameters

**Phosphate solubilization:** The qualitative assay recorded the solubilization of inorganic phosphate in fresh bacterial culture. The strains were grown on a tri-calcium phosphate-minimal agar media (NBRIP, National Botanical Research Institute phosphate) for three days (72 h) at 30°C. The emergence of a clear zone surrounding the bacterial colonies indicated phosphate solubilization (Li *at al*., 2018; Paul & Sinha, 2017).

**Production of IAA:** Indole Acetic Acid (IAA) production was assessed using the method outlined by Rashid *et al*., (2012). Endophytic bacteria were cultured in TSB with 0.2% L-tryptophan and incubated for five days at 37°C with shaking at 150 rpm. The cell-free supernatant was assessed for IAA synthesis using 0.5% Salkowski reagent, resulting in a pink-red color post-centrifugation at 12,000 rpm for 10 min. IAA was quantified by using a spectrophotometer to measure absorbance at 530 nm, and a standard curve of IAA was used to compute the IAA concentration in μg/ml.

**Ammonia production:** To evaluate bacterial ammonia production, 10 mL of recently cultured bacterial strains were placed in test tubes containing peptone water and incubated for 48-72 h at 30°C. Upon adding 0.5 mL of Nessler's reagent to the culture, the media's color changed from yellow to brown, showing ammonia production (Marques *et al*., 2010).

**Hydrolytic enzyme production:** Hydrolytic enzyme production was determined by examining the proteolytic activity of bacterial isolates. Endophytic bacteria were streaked on skim milk agar medium and left to grow at 37°C for 24-48 h. The presence of a clear hollow zone surrounding the bacterial colonies indicated proteolytic activity. (Adinarayana *et al*., 2003).

**Gnotobiotic canola root elongation assay:** The method outlined by Penrose & Glick (2003) for evaluating the capacity of endophytic bacteria to enhance root growth. Canola seeds were obtained from the local market Islamabad. Bacterial-inoculated seeds and control seeds (uninoculated) were placed on filter paper plates and placed in a plant growth chamber. The chamber maintained controlled conditions including a constant temperature of 25°C, 12 h light/dark cycles, and a relative humidity of 60%. After five days, the lengths of the plantlets' roots were measured for analysis.

**Extraction of secondary metabolites:** Endophytic bacteria were grown in TSA at 30°C and 120 rpm for 48 h. The resulting culture was centrifuged at 10,000 rpm for 10 min to separate the pellet and supernatant. The pellet was dissolved in methanol, incubated for 24 h, and then sonicated for 30 min with 5 min intervals. After centrifugation, the supernatant (A) was collected in a falcon tube. The same process was repeated for the remaining pellet using methanol, resulting in supernatant (B). Combining solvents, A and B, a crude extract of bioactive metabolites was obtained and evaporated at room temperature. Finally, dimethyl sulfoxide (DMSO) was used to dissolve the extract for further analysis (Rehman *et al*., 2017).

**Biological evaluation**

**Total phenolics:** The total phenolics content was determined using the protocol outlined in Singleton *et al*., (1999), which involves utilizing the Folin–Ciocalteau reagent. Absorbance measurements were taken at 700 nm, and the total phenolics content was estimated by referencing it to the gallic acid standard curve.

**Total flavonoids:** Colorimetric technique was used to evaluate the total flavonoid content outlined by Zhishen *et al*., (1999), with absorbance readings recorded at 510 nm. Flavonoid contents were expressed in milligrams of quercetin equivalent per gram of extract, determined through a calibration curve based on quercetin.

**DPPH free radical scavenging assay:** The approach utilized by Tai *et al*., (2011) was adapted with minor changes to estimate the scavenging activity of free radicals. Different concentrations (100, 50, 25, and 12.5 µg/mL) of crude extract bacterial endophytes were distributed across 96-well plates, with a final volume of 200 µl achieved by adding DPPH to each well. Ascorbic acid served as the positive while DMSO served as the negative control. After incubating the samples for 1 h at room temperature, absorbance was taken at 630 nm using a microplate reader. IC50 values were expressed as µg AAE/mg of extracts. The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ RSA} = [1 - (\text{OD of Extract})/(\text{OD of Control})] \times 100$$

**Total antioxidant capacity (TAC):** The total antioxidant capacity was assessed through the phosphomolybdenum method (Prieto *et al*., 1999). Samples (4 mg/mL) were combined with phosphomolybdenum reagent, incubated at 95°C for 90 min, then cooled and transferred to 96-well plates. Positive control was ascorbic acid and negative control was DMSO. Absorbance was measured at 630 nm using a microplate reader. Results were reported as µg of ascorbic acid equivalents per mg of extract (µg/mg).

**Total reducing power:** The passage outlines a method for assessing the total reducing power of test samples using a procedure based on Oyaizu *et al*., (1986), with minor adjustments. The test samples, derived from a stock solution at a concentration of 4 mg/mL, are incubated in eppendorf tubes after the addition of phosphate buffer and potassium ferricyanide. Following incubation, trichloroacetic acid is introduced, and the resulting mixture is centrifuged. Supernatant from each centrifuged sample is transferred to a microplate, combined with ferric chloride solution, and thoroughly mixed. A microplate reader is used to measure absorbance at 630 nm. Results, expressed as µg AAE (ascorbic acid equivalent) per mg of extracts, are calculated based on triplicate analyses. Ascorbic acid and DMSO are used as the positive and negative controls, respectively.

**Detection of bioactive compounds:** Bioactive compounds were detected in the organic extract of bacterial strain RBL4 using GC-MS analysis, following the methodology outlined by Refish *et al*., (2016). The experiment employed a GC-MS instrument (QP2010 Ultra, Shimadzu Europa GmbH, Germany) with an RTX-5MS column (30 × 0.25 × 0.10 m). The temperature increased at the rate of 3°C $min^{-1}$, with initial and final temperatures set at 100°C and 250°C, respectively. Peaks in the chromatograph were provisionally identified as bioactive compounds through comparison with the NIST library.

**Statistical analysis**

All the spectrophotometric determinations were conducted in triplicate; ANOVA was used using OriginPro 2024. Values labeled with different letters show significant differences ($p<0.05$).

**Results**

**Isolation and identification:** Bacterial endophytes from *B. lycium* roots were identified through 16S rRNA gene sequence analysis. The selected endophytic bacteria for the current study belonged to genus *Bacillus*. Bacterial strains are given in Table 1, along with the closest-matching strain, similarity index, and accession numbers (Table 2 and Fig. 1).

**Beneficial plant traits of endophytic bacteria:** The plant growth promoting traits of these strains was assessed based on four parameters. The isolates' ability to solubilize inorganic phosphate from the medium was evaluated. Our findings indicate that all the bacterial isolates solubilized phosphate. Bacterial isolates RBL1 and RBL4 strains were the best phosphate solubilizers. In this study, it was seen that all endophytic bacteria could potentially be able to produce ammonia except RBL5. Most isolates demonstrated the ability to produce IAA regardless of the presence of tryptophan. However, the addition of tryptophan notably enhanced IAA production, indicating its involvement in the biosynthetic pathway. IAA concentrations supplemented with tryptophan ranging from 1.19 to 3.35 µg/mL, whereas those without tryptophan ranged from 0.03 to 1.38 µg/mL. A clear zone around the colonies indicated a positive result for protease enzyme activity. Bacterial isolates RBL1, RBL3, and RBL4 exhibited positive protease enzyme activity (Table 3).

**Effect of endophytic bacteria on growth canola seeds:** Inoculating canola seeds with endophytic isolates such as *B. subtilis* and *B. pseudomycoides* significantly increased the root length of seedlings compared to those in the control group (Table 4). The inoculation of *B. subtilis* in canola plant resulted in the increased root length by 10.52cm as compared to control. Variations may arise in the plant-endophyte relationship due to differences in genetic composition.

**Table 1. Colony morphology of the selected bacterial isolates from *B. lycium*.**

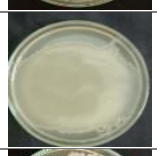
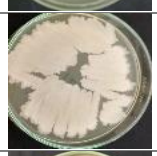
| Isolates | Images | Color | Form | Size (µm) | Texture | Elevation | Margin | Gram staining |
|---|---|---|---|---|---|---|---|---|
| RBL1 |  | Yellow | Round | 1-2 | creamy | Raised | Undulate | + |
| RBL2 |  | White | Round | 3-5 | Dry | Raised | Entire | + |
| RBL3 |  | Off- White | Irregular | 1-3 | Slightly creamy | Flat | Entire | + |
| RBL4 |  | Off-white | Irregular | 0.7-0.8 | Dry, Sticky | Flat | Undulate | + |
| RBL5 |  | Light yellow | Round | 0.8-0.9 | Dry | Slightly raised | Entire | + |

**Table 2. Bacterial isolates used in this study.**

| Strain ID | The closest match in NCBI database | Similarity index (%) | Accession no. |
|---|---|---|---|
| RBL1 | *Bacillus paramycoides* | 100 | PP231775 |
| RBL2 | *Bacillus pseudomycoides* | 99.73 | PP231776 |
| RBL3 | *Bacillus wiedmannii* | 100 | PP231777 |
| RBL4 | *Bacillus subtilis* | 100 | PP231778 |
| RBL5 | *Calidifontibacillus erzurumensis* | 100 | PP231779 |

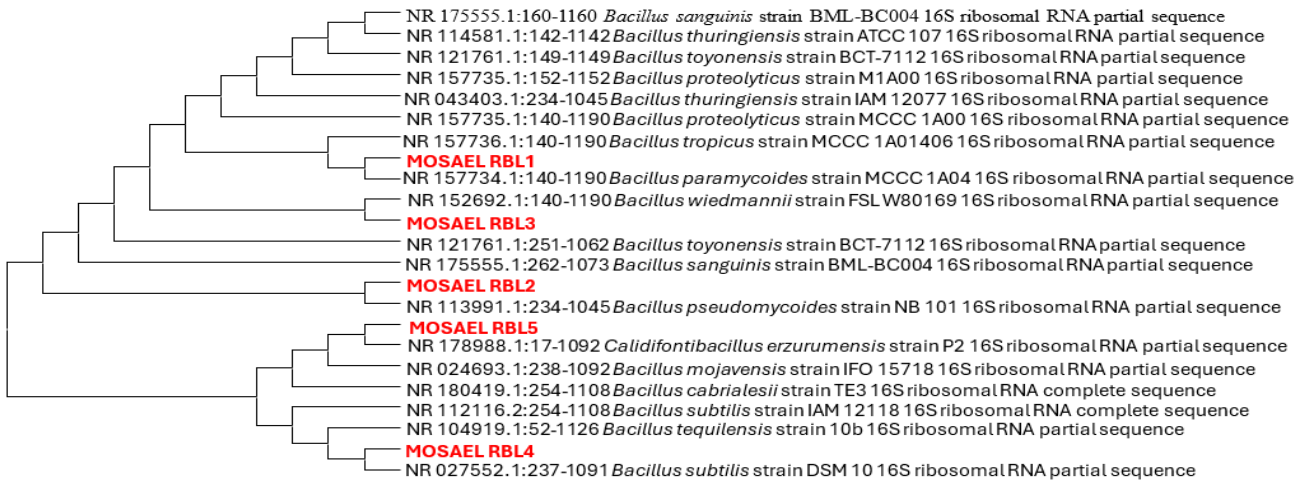



Fig. 1. Phylogenetic analysis of isolated bacterial strains with reference strains from NCBI.

**Table 3. Plant growth promoting traits of endophytic bacteria.**

| Strain ID | Strain name | PGB traits | | | | |
|---|---|---|---|---|---|---|
| | | P-solubilization | Ammonia production | IAA production | | Protease |
| | | | | Without tryptophan | With tryptophan | |
| RBL1 | *Bacillus paramycoides* | ++ | + | 1.23 | 3.19 | + |
| RBL2 | *Bacillus pseudomycoides* | + | + | 1.38 | 3.16 | – |
| RBL3 | *Bacillus wiedmannii* | + | + | 0.03 | 1.25 | + |
| RBL4 | *Bacillus subtilis* | ++ | + | 0.33 | 3.35 | + |
| RBL5 | *Calidifontibacillus erzurumensis* | + | – | 0.17 | 1.19 | – |

The "+" symbol indicates positive activity, while the "–" symbol denotes no activity. "+" represents a small zone with a diameter <10 mm, "++" indicates a medium diameter ranging from 10 to 20 mm, and "+++" signifies a diameter > 20 mm

**Table 4. Effect of bacterial isolates on Canola root length.**

| Sr. No. | Bacterial ID | Bacterial strains | Average root length (cm) |
|---|---|---|---|
| 1. | Control | | 4.5 ± 0.22$^{d}$ |
| 2. | RBL1 | *Bacillus paramycoides* | 8.91 ± 0.44$^{b}$ |
| 3. | RBL2 | *Bacillus pseudomycoides* | 7.91 ± 0.34$^{cd}$ |
| 4. | RBL3 | *Bacillus wiedmannii* | 8.32 ± 0.42$^{bc}$ |
| 5. | RBL4 | *Bacillus subtilis* | 10.52 ± 0.67$^{a}$ |
| 6. | RBL5 | *Calidifontibacillus erzurumensis* | 5.97 ± 0.64$^{e}$ |

## Biological evaluation

**Determination of total flavonoid and phenolic contents:** A wide range of flavonoids concentrations are present in the methanolic bacterial crude extracts [Figs. 2A, 3a (Standard curve)]. RBL4 exhibits the greatest flavonoid concentration of any bacterial extract, with 58.5 µg QE /mg, followed by RBL2 with 52.31 µg QE/mg. The lowest phenolic content was shown by RBL3. The overall phenolic content of bacterial crude extracts varies widely. The results showed a range of 62.9 to 180.1 µg GAE/mg of extract. In our investigation, the extract of RBL1 had the greatest concentration of Gallic acid equivalent phenols (183.1 µg GAE/mg), followed by RBL4. In contrast, RBL2, RBL3 and RBL5 had significantly lower phenol concentrations [Figs. 2B, 3b (Standard curve)].

**Total antioxidants capacity:** Out of all the bacterial extracts, it was observed that RBL1 exhibited the highest level of antioxidant activity with a value of 170.1 µg/mg. RBL4 and RBL3 were found to have total antioxidant capacities of 155 and 119 µg/mg, respectively [Figs. 2C, 3c (Standard curve)].

**Reducing power:** In the reducing power assay, the ability to reduce was evaluated by changing from $Fe^{3+}$ to $Fe^{2+}$. The ability of extracts to reduce antioxidants was examined. The results for RBL4 and RBL1 showed that they were excellent electron donors, capable of stabilizing free radicals and having the maximum reducing power (196 and 152 µg AAE/mg of extract, respectively) [Figs. 2D, 3d (Standard curve)].

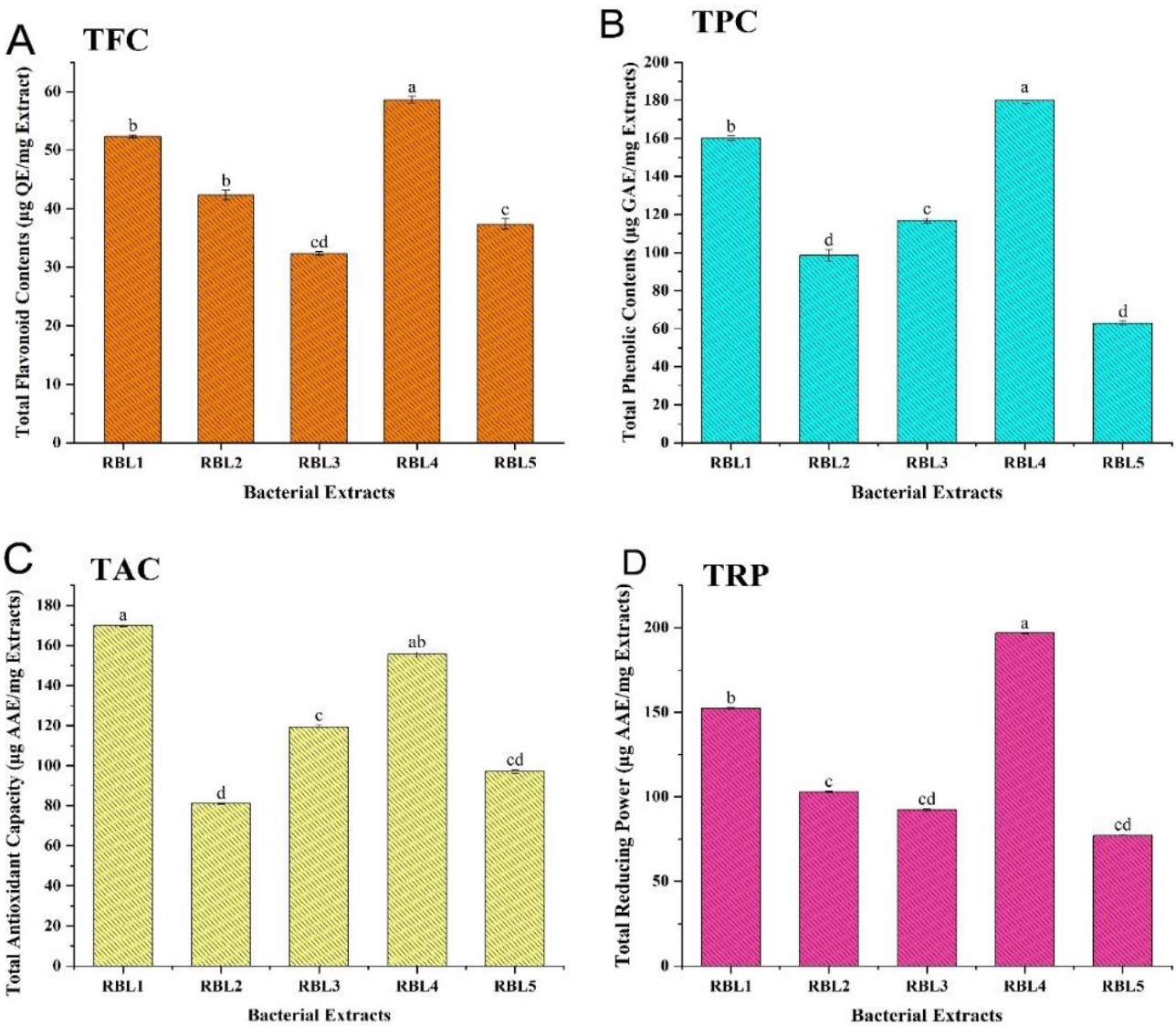



Fig. 2. A Total Flavonoid Content, B Total Phenolic Content, C Total Antioxidant Capacity, D Total Reducing Power. Differences marked by distinct lowercase letters indicate statistical significance ($p<0.05$).

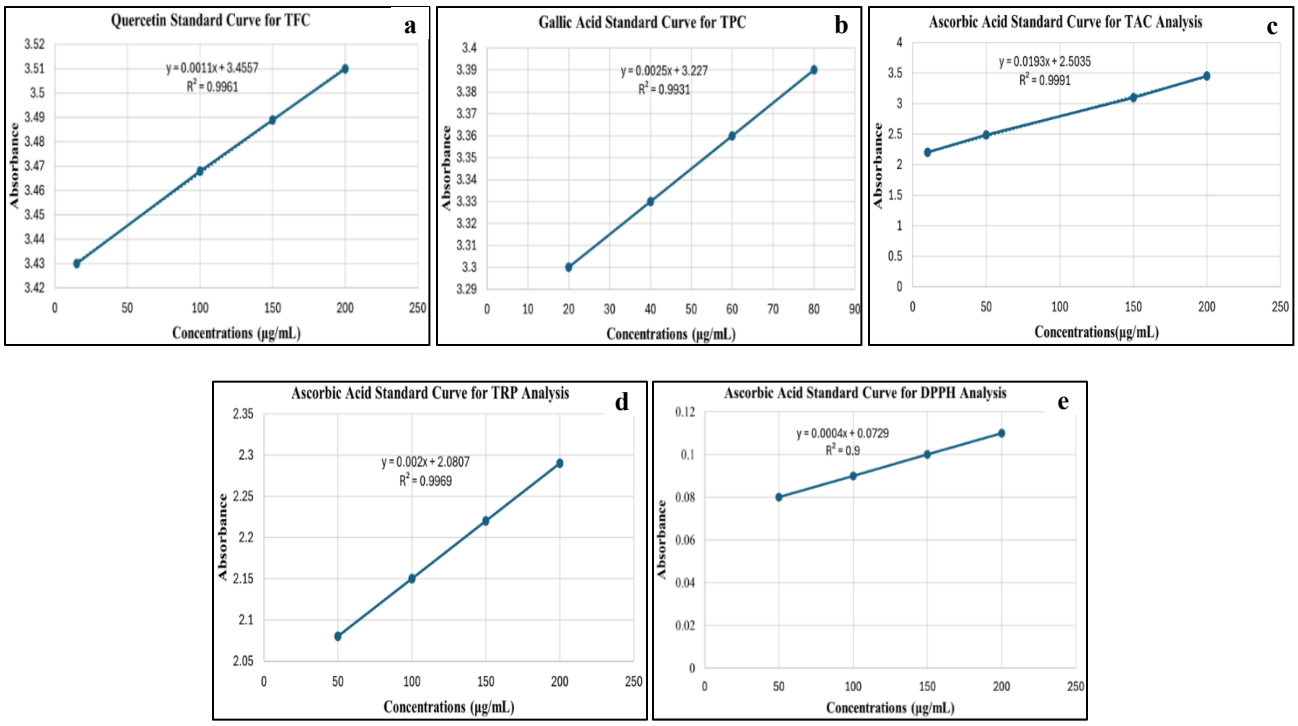



Fig. 3. Standard curves(a-e): a. Quercetin calibration curve for determining TFC. b. Gallic acid calibration curve for estimating TPC. c. Ascorbic acid calibration curve for analyzing TRP. d. Ascorbic acid calibration curve for estimating TAC. e. Ascorbic acid calibration curve for evaluating DPPH RSA activity.

**DPPH:** The DPPH reagent was used to assess the percentage of free radical scavenging activity (%RSA) of endophytic bacteria. This was observed through a visible color transition indicating reduction of the unstable DPPH molecule to a stable form due to antioxidant action. Evaluation involved determining the half maximal inhibitory concentration (IC50). RBL4 exhibited the highest scavenging activity with an IC50 value of 36.8 µg/mL, while RBL1 and RBL2 showed IC50 values of 85.1 and 112, respectively. Table 5 presents the %RSA of each bacterial extract compared to ascorbic acid as the standard [Standard curve, Fig. 3e].

**GC-MS analysis:** Mass spectrometry analysis of RBL4 revealed predominantly saturated β-fatty acids ranging from $C_8$ - $C_{24}$ in chain length, constituting the majority of identified compounds (Table 6). The primary fatty acid detected was 9-Hexadecanoic acid, comprising 46.57% of the oil, followed by benzene at 15.56%. Other compounds identified include 9,12-Octadecadienoic acid, Octane, Ethylbenzene, Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, 9-Octadecenoic acid, and Pentafluoropropionic acid.

**Table 5. DPPH free radical scavenging assay endophytic isolates with IC50 values.**

| Strain ID | Strain name | 400 µg/mL | 300 µg/mL | 200 µg/mL | 100 µg/mL | IC50 Value |
|---|---|---|---|---|---|---|
| RBL1 | *Bacillus paramycoides* | 56.77 | 54.66 | 52.55 | 50.25 | 85.1 |
| RBL2 | *Bacillus pseudomycoides* | 60.9 | 56.8 | 52.8 | 49.9 | 112 |
| RBL3 | *Bacillus wiedmannii* | 55.13 | 48.57 | 46.31 | 44.38 | 290 |
| RBL4 | *Bacillus subtilis* | 78.1 | 72.9 | 66.8 | 52.6 | 36.8 |
| RBL5 | *Calidifontibacillus erzurumensis* | 56.47 | 48.32 | 44.09 | 39.01 | 303 |

**Table 6. GC-MS analysis of bioactive compounds produced by crude extract of *B. subtilis*.**

| No. | Retention time | Compounds detected | Similarity index (%) | Peak area (%) | Molecular formula |
|---|---|---|---|---|---|
| 1. | 3.041 | Pentafluoropropionic acid | 83 | 2.39 | $C_{10}H_{15}F_5O_2$ |
| 2. | 3.118 | Octane | 96 | 4.51 | $C_8H_{18}$ |
| 3. | 4.324 | Ethylbenzene | 97 | 3.76 | $C_8H_{10}$ |
| 4. | 4.500 | Benzene | 98 | 15.56 | $C_8H_{10}$ |
| 5. | 4.967 | 9-Octadecenoic acid | 95 | 5.13 | $C_{19}H_{36}O_2$ |
| 6. | 21.934 | 9,12-Octadecadienoic acid | 96 | 13.41 | $C_{19}H_{34}O_2$ |
| 7. | 21.996 | 9- Hexadecanoic acid | 93 | 46.57 | $C_{17}H_{32}O_2$ |
| 8. | 25.901 | 1,2-Benzenedicarboxylic acid | 94 | 3.12 | $C_{24}H_{38}O_4$ |

## Discussion

Numerous bioactive substances with medicinal potential have been discovered within the endophytes of medicinal plants such as tannins, terpenoids, quinones, steroids, quinones, alkaloids, saponins and phenolic acids. Scientists have identified and characterized these compounds using a combination of traditional and advanced techniques. These bioactive molecules serve as a baseline for further research aimed at developing and refining therapeutic compounds with diverse applications in healthcare and beyond (Younis *at al*., 2022). Their discovery opens new avenues for improving treatments and addressing various challenges in fields such as medicine, agriculture, and environmental protection (Nazim & Bano, 2024).

Medicinal plants often host endophytic bacteria that are closely linked to their production of secondary metabolites and therapeutic properties. In this study, we focused on endophytic bacteria isolated from *B. lycium*, a plant of significant ethno-botanical importance. Research suggests that the medicinal properties of a plant are often more closely associated with its endophytic community than with its own biochemical makeup (Iqrar *et al*., 2021). To explore this connection, we conducted various biological assays to assess the potential medicinal properties of bacteria associated with *B. lycium*. Through analysis of the 16S rRNA gene sequence, we identified endophytic bacteria belonging to genus *Bacillus*. These findings shed light on the potential role of bacterial isolates in contributing to plant growth promotion.

It is believed that endophytic bacteria directly influence the fitness and growth of plants. Phosphate solubilization is seen as one of the direct methods for enhancing growth (AlKahtani *et al*., 2020). In our study, most of the isolates could solubilize insoluble phosphate. Following our findings, Afzal *et al*., (2017) discovered that all the plant growth promoting bacteria could be P. solubilized. According to Marques *et al*., (2010), the amount of IAA produced by bacteria can favor plant growth. In line with other studies, we discovered that all bacterial isolates without tryptophan produce IAA and substantially more of it when tryptophan is present. According to Numan *et al*., (2022), biological control agents work alongside enzymes, including cellulases, proteases, and chitinases, to combat phytopathogenic fungi. Most bacterial strains in our investigation exhibit activity for protease enzymes. In this study, canola seeds inoculated with the five potent bacterial endophytes showed improved root length as compared to uninoculated seeds. These findings align with Hassan (2017), who claimed that adding *B. subtilis* Tp.6B and *B. cereus* Tp.1B to maize seeds increased the weight and length of roots compared to the control group.

In biological systems, oxidation naturally occurs, generating reactive peroxyl and hydroxyl radicals that can harm DNA, cell membranes, and proteins, potentially leading to diseases. Extract from *B. subtilus* and *B. wiedmannii* demonstrate the highest total antioxidant activity, indicative of their ability to scavenge free radicals and donate electrons. Endophytic bacteria isolated from plants like *Centella asiatica* display significant reductive potentials, contributing to their ability to stabilize free radicals (Rafat *et al*., 2012). *B. subtilis* and *B. paramycoides* extracts exhibit the highest reducing power, likely due to their electron-donating capacity, which correlates with their phenolic content and antioxidant potential. These results align with earlier studies, highlighting the importance of flavonoids in combating lipid oxidation. The study's results align with similar research on endophytic bacteria associated with other plant hosts, emphasizing *B. subtilis* effectiveness as an antioxidant agent.

Antioxidants play a key role in neutralizing free radicals, preventing cellular damage. The DPPH free radical scavenging assay is a method to assess antioxidant activity, with methanolic extracts from endophytic bacteria demonstrating effectiveness in reducing the stable radical DPPH. Particularly, extract from *B. subtilis* exhibited notable scavenging activities compared to the control (Ascorbic acid equivalent). The IC50 value serves as a measure of antioxidant potency, with lower values indicating higher activity. These findings align with previous research on endophytic bacteria associated with medicinal plants (Nongkhlaw & Joshi, 2015)

GC-MS analysis of *B. subtilis* findings shows a variety of free and bound fatty acids, resembling those documented by Ibrahim *et al*., (2013). Fatty acid composition in these lipopeptides depends on the growth medium. High levels of hexadecanoic acid suggest its significance in bacterial growth (Guo *et al*., 2012).

## Conclusion

The current study proposes that endophytes have the potential to serve as a valuable source of secondary metabolites other than host plants. Our current investigation proved that all isolated endophytic bacteria exhibit plant growth promoting potential. We conclude significant observation of biological activities shown by bacterial strains associated with *B. lycium*. Additionally, they may enhance plant nutrient absorption, particularly in harsh environmental conditions Therefore, we recommend further studies on evaluating the use of these endophytic bacteria specifically *B. subtilis* in field trials for canola and other crops to facilitate the development of a commercially viable product.

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