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Original article

Therapeutic prospects of endophytic *Bacillus* species from *Berberis lycium* against oxidative stress and microbial pathogens

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ABSTRACT

Endophytes are microorganisms residing within plant tissues. Bacterial endophytes are important sources for production of pharmaceutically important metabolites. *Berberis lycium* is an important medicinal plant and there exist no report regarding isolation and determination of bioactive potential of its bacterial endophytes. Therefore the present study was aimed to isolate and identify bacterial endophytes from *Berberis lycium*. The study resulted in isolation of 20 strains of bacterial endophytes. Based on their antibacterial activity three strains were identified as *Bacillus cereus* (LBL6), *Bacillus thuringiensis* (SBL3) and *Bacillus anthracis* (SBL4) on basis of 16SrRNA gene using universal primers. Crude ethyl acetate extracts of LBL6, SBL3 and SBL4 were further evaluated for antioxidant and antifungal activities. Moderate antioxidant activity (56 %) at a concentration of 1000 μg/mL was observed for LBL6 followed by 45 and 43 % activity by SBL4 and SBL3 respectively. Significant antifungal activity was observed against *Aspergillus niger* (60 %) and *Aspergillus flavus* (56 %) at concentration of 4 mg/mL of SBL3 and SBL4 respectively. GCMS analysis of extract (LBL6) exhibited presence of 12 bioactive secondary metabolites corresponding to antimicrobial, antifungal, antioxidant, antitumor and anticancer activities. In conclusion, present study highlighted the importance of *Berberis lycium* to host diverse bacterial endophytes of pharmaceutical importance.

1. Introduction

Natural products and metabolites are chemical compounds isolated from different living organisms. Majority of higher plants produce extractable organic compounds that are being used as chemical raw materials for many industrial, technical, commercial and scientific purposes (Zhang et al. 2018a,b). Natural products are widely used in human therapy, agriculture, veterinary and scientific research. Natural products containing microbial metabolites are utilized in medicine, agriculture, for chemical synthesis of various new analogs in drug design. Medicinal plants are also important sources being used for centuries due to their therapeutic potential. Many important compounds used as drugs today were once originated from plants (Bibi et al. 2011).

Bacterial endophytes colonize in internal plant tissues and form different associations with plants such as mutualistic, trophobiotic, commensalistic and symbiotic (Esmaeel et al. 2016, Esmaeel et al. 2018). Bacterial endophytic are diverse in nature as both Gram negative and Gram positive bacteria have been isolated and identified from various tissues of plants (Zinnielet al. 2002). Many endophytic organisms belong to bacterial genera commonly present in soil including Bacillus, Burkholderia and Pseudomonas. According to previous reports endophytes are responsible for production of biotechnologically important bioactive compounds within plants (Parthasarathi et al. 2012; Joseph and Priya, 2011;

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Owen and Hundley, 2004). Many compounds have been identified from bacterial endophytes including cryptocin, taxol, cryptocandin, oocydin, jesterone, pseudomycins, isopestacin and ambuic acid (Strobel, 2002). Secondary metabolites isolated from endophytic bacteria are of biological significance having anticancer, antibacterial, insecticidal, antiviral and antifungal activities (Ryan et al. 2008).

Berberis lycium is an evergreen shrub belonging to family Berberidaceae. It is native to Pakistan and used as food and folk medicine. The different plant parts such as bark, leaves, fruits, roots and stem are used as food or medicine by people. The plant root is called as "Darhald" and it is used as diaphoretic and for treatment of bleeding piles (Khan et al. 2010). *Berberis lycium* produces many important extractable bioactive compounds namely berbamine, berberine, chinabine, palmatine, karakoramine, chinabine acetic acid, ascorbic acid and maleic acid (Ahmed et al. 2017). Laxative and cool fruit of *Berberis lycium* is used for pharyngitis, typhoid fever and intestinal colic. *Berberis lycium* is important in treatment of cholera, dysentery, eye troubles, leprosy and diarrhea (Gupta et al. 2015). Many important disorders like liver and abdominal, skin problems, cough and bacterial dysentery are successfully treated by using *Berberis lycium*. It also has hyperlipidemic, antipyretic, hypoglycemic, hepato-protective and anticarcinogenic properties (Mustafa et al. 2020).

Berberis lycium has not been studied for the isolation of bacterial endophytes and their bioactivities. In present study we determined the diversity and bioactivities of culturable bacterial endophytes inhabiting *Berberis lycium*.

2. Materials and methods

Experiments were conducted in Plant Microbiology lab at Microbiology Department, University of Haripur, Pakistan.

2.1. Plants collection, identification and surface sterilization

Plants of *Berberis lycium* were collected from Marghazar, Swat and Murree in summer season and brought to the laboratory at the same day for the isolation of endophytic bacteria (Fig. 1). The plants were confirmed taxonomically as *Berberis lycium* by experts at of Botany Department, Hazara University, Mansehra Pakistan.

Fig. 1. Vegetative and reproductive growth of *Berberis lycium*.

2.2. Isolation and development of pure culture of endophytic bacteria

The collected plant leaves and stems were cut into 2–3 cm long pieces and washed under running tap water. These pieces were then sterilized using distilled water and soaking in 70 % ethanol for 30 s. After that washing and soaking was done with sterile water and 2 % sodium hypochlorite solution for 1 min respectively. Finally, pieces were rinsed extensively in sterile water and drained. The drained segments were than inoculated on Petri dishes and incubated for 24 hrs at 28 °C that resulted in emergence of bacterial growth from cut ends. Based on the morphological appearance of the bacterial colonies, they were randomly picked and subcultured. Colonies were purified after a few cycles of subculturing and were finally preserved in glycerol stocks and stored at −20 °C till further use.

2.3. Microscopy and biochemical evaluation

Gram staining was done to observe bacterial isolates under microscope. Microscopic observation was carried under oil immersion lens to observe morphological appearance of bacterial strains. According to Bergey's Manual various biochemical tests catalase, oxidase and gelatin hydrolysis test were performed for biochemical characterization of selected isolates.

2.3.1 Catalase test

A small amount of bacterial colony was picked with a sterile loop and applied to the clean glass slide. A drop of 3% hydrogen peroxide (H_2O_2) was added to the slide and mixed well. A positive result was indicated by the formation of bubbles.

2.3.2 Oxidase test

A piece of filter paper was soaked with freshly made 1 %solution of the reagent (tertramethyl-p-phenylene-diamine-dihydrochlor ide). Then a small amount of bacterial colony was picked up with sterile loop and rubbed on to the filter paper. A positive result was indicated by the appearance of deep purple color.

2.3.3 Gelatin hydrolysis test

Small amount of culture was taken and inoculated on to the nutrient gelatin medium by stabbing4 to 5 times half inch into the medium. Now the test and an un-inoculated tube were incubated for 24–48 h at 37C. The tubes were gently removed from the incubator and placed in refrigerator until the control tube solidifies. Then the tubes were inverted to detect the liquefaction by the organism.

2.4. Extraction of secondary metabolites

Each of the isolated bacteria was inoculated in Erlenmeyer flask containing nutrient broth media and the culture flasks were then incubated on shaking incubator at 110 rpm for 7 days at room temperature. After 7 days flasks were removed from the rotary shaker and ethyl acetate was added to the cultured broth and left overnight. Next day, filtration was carried out using micro-filters to remove the bacterial cells from the extracted broth. After filtration, culture broth was extracted with ethyl acetate using a separating funnel. Rotary vacuum chamber was used to obtain the dry form of extracts. As dried forms of extracts were obtained. Crude extract (5 mg/mL) was dissolved in DMSO and used for further assays.

2.5. Antibacterial activity

Crude ethyl acetate extracts (1000 μg/mL) were used for screening of antibacterial activity using disc diffusion method. Antibacterial activity was tested against seven ATCC strains (*Escherichia coli*

ATCC 25922, *Bacillus spizizenii* ATCC 6633, *Staphylococcus aureus* ATCC 292013, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Listeria monocytogenes* ATCC 35152, and *Salmonella typhimurium* ATCC 14028). The ATCC strain's cultures were diluted in sterilized distilled water. Then, the inoculum was seeded with cotton swabs on nutrient agar plates. The samples were impregnated on sterile paper discs and were placed on nutrient agar which was seeded with ATCC strains. DMSO and chloramphenicol were used as negative and positive control respectively. The plates were incubated at 37 °C and zones of inhibition were measured after 24 hrs of incubation.

2.6. Molecular identification of endophytic bacteria

DNA of bacterial strains exhibiting significant antibacterial activity was extracted by using "Nucleo spin Microbial DNA" kit following manufacturer's instruction. For PCR amplification, 1 μL of diluted 16SrRNA gene universal primers (Fd1 5'-AGAGTTT GATCCTGGCTCAG-3' and Cd1 5'-AAGGAGGTGATCCAGCC-3') and Ampliqon (Taq polymerase) (Thermo fisher scientific) were used to make the final reaction mixture. The amplification was done by preliminary denaturation at 95 °C for 5 min followed by 35 cycles of amplification, denaturation (95 °C, 40 sec), annealing (51 °C, 30 sec), elongation (72 °C, 1 min and 30sec) and a final elongation (72 °C, 10 min). Purification of PCR product and sequencing was done by commercially available services of Alpha Genomics, Pakistan.

2.7. Phylogeny of isolated bacterial endophytes

For phylogenetic analysis of the isolated bacterial endophytes, closely related sequences were retrieved from NCBI and Clustal W was used to perform multiple sequence alignment. Then mega format of aligned files were exported and phylogenetic tree was constructed in MEGAX using neighbor-joining method. Default parameters were selected for construction with bootstrap values of 1000.

2.8. Antioxidant activity

The DPPH free radical scavenging assay was used for determination of antioxidant potential of LBL6, SBL3 and SBL 4 (Kandasamy et al. 2015).The antioxidant potential of the crude extracts was judged by monitoring their capacity to quench the stable 2, 2-diphenyl 1-picrylhydrazyl (DPPH) free radical. 0.15 mL of different concentrations of samples (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL and 62.5 μg/mL were added with 2.85 mL DPPH reagent (1 mg of DPPH reagent/25 mL of dimethyl sulfoxide) in falcon tubes and incubated in dark for 30 min. The absorbance was noted at 517 nm by using UV–Vis spectrophotometer (Arokiyaraj et al. 2008). Different concentrations of ascorbic acid were used as positive control, while DMSO was used as negative control. DPPH free radical-scavenging activity was calculated by using formula

DPPH RSA (%) inhibition = [(control OD − sample OD) / control OD)] × 100

Where:

Control OD = absorbance without sample

Sample OD = absorbance in the presence of the sample

2.9. Antifungal activity

Two fungal strains *Aspergillus niger* and *Aspergillus flavus* were used to screen antifungal potential of ethyl acetate extracts of LBL6, SBL3 and SBL4 by agar tube dilution method as described by Bibi et al. (2012). SDA agar media (5 mL) was dispensed in screw caped tubes and autoclaved. The tubes were then left for cooling and before solidification 200 μL (400 μg/mL) of bacterial extracts were added. Sodium benzoate (2.4 μg/mL) and pure DMSO were used as positive and negative control respectively. The tubes were shaken well and allowed to solidify in the slanting position. In respective tubes, 4 mm of 7 days old fungal culture was inoculated. Then, the tubes were incubated at 27 °C for 7 days. The growth inhibition was calculated by using following formula;

Percentage Inhibition of Fungal growth = (Lgc - Lgt) / Lgc × 100

Where:

Lgt = linear growth of test sample

Lgc = linear growth of control

2.10. GC–MS analysis of bacterial extract

The crude ethyl acetate extract of LBL6 was further evaluated for its chemical composition using GCMS (Perlin Elmer Clarus 600, Gas Chromatograph, Elit 5 MS column). A 20 min run was conducted from initial temperature of 40 °C to final temperature of 250 °C. Mass spectrum was recorded in the range of 40–600 *m/z*. Various compounds as eluted through the GC column resulted in generation of peaks. These peaks along with retention time were recorded and data was correlated with mass spectra of previously reported compounds. Database was explored for compounds with same retention time and molecular mass. Bioactivities of previously reported natural compounds were also documented and compared to correlate the activities of bacterial extract and its constituents.

2.11. Statistical analysis

All the bioassay experiments were conducted in triplicates and results are expressed as mean with standard deviation. One way analysis of variance was used to analyze data using IBM SPSS for Windows, Version 20. Results with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Endophytic bacteria from Berberis lycium

As a result of inoculation of plant material on nutrient agar different strains of bacteria were isolated from stems and leaf sections of *Berberis lycium*. A total of 20 different bacteria were isolated, 13 from stem and 7 from leaves (Fig. 2).

3.2. Biochemical characterization of isolated bacterial endophytes

After obtaining the pure culture of bacterial endophytes, Gram staining and biochemical tests were performed. Gram staining and microscopy of bacterial endophytes revealed 18 strains as Gram-positive bacilli while 2 were Gram-negative bacilli. As a result of biochemical analysis all the isolates showed positive results for catalase and negative for oxidase test while in case of gelatin hydrolysis, 18 isolates show positive results and 2 isolates showed negative results (Table 1 & Figs. 3 & 4).

3.3. Antibacterial activity of endophytic bacteria

Antibacterial activity of crude ethyl acetate extracts of endophytic bacteria were determined against 7 ATCC bacterial strains. The results indicated that extract of LBL6 was most effective, exhibiting a zone of inhibition ranging from 19 to 8 mm with significant effect against *Pseudomonas aeruginosa*, *Bacillus spizizenii*, *Salmonella typhimurium* and *Klebsiella pneumoniae*. Crude extract of SBL3 and SBL4 exhibited best activity against *Escherichia coli*

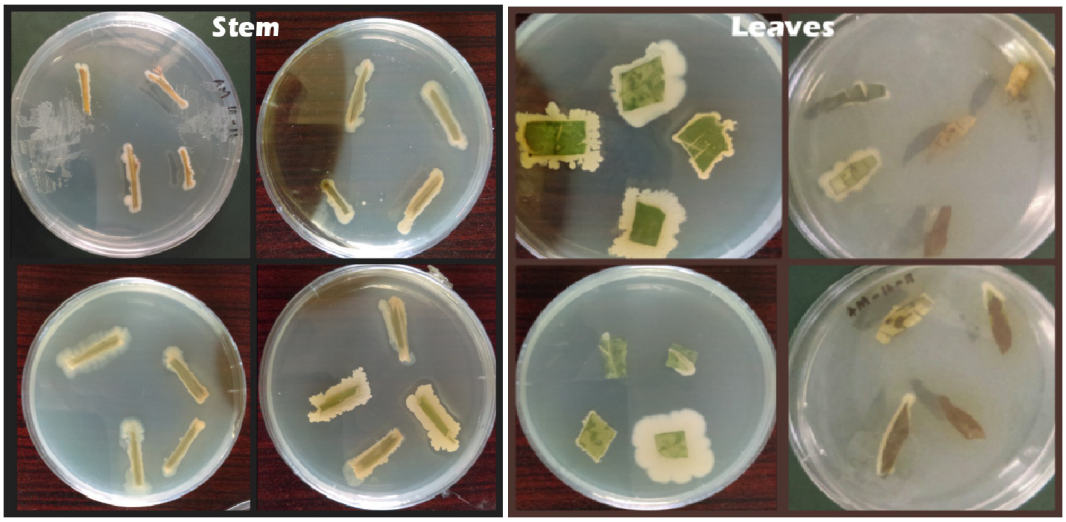

Fig. 2. Initiation of endophytic bacterial growth from stem and leaves.

Table 1
Morphological and Biochemical characterization of isolated endophytic bacterial strains of *Berberis lycium.*

Endophytic bacterial isolates	Gram's stain	Shape	Catalase	Oxidase	Gelatin hydrolysis
SBL1	- ve	Rod	+ve	- ve	+ve
SBL2	+ve	Rod	+ve	- ve	+ve
SBL3	+ve	Rod	+ve	- ve	+ve
SBL4	+ve	Rod	+ve	- ve	+ve
SBL5	+ve	Rod	+ve	- ve	+ve
SBL6	+ve	Rod	+ve	- ve	+ve
SBL7	+ve	Rod	+ve	- ve	+ve
SBL8	+ve	Rod	+ve	- ve	+ve
SBL9	+ve	Rod	+ve	- ve	+ve
SBL10	- ve	Rod	+ve	- ve	+ve
SBL11	+ve	Rod	+ve	- ve	+ve
SBL12	+ve	Rod	+ve	- ve	+ve
SBL13	+ve	Rod	+ve	- ve	+ve
LBL1	+ve	Rod	+ve	- ve	+ve
LBL2	+ve	Rod	+ve	- ve	- ve
LBL3	+ve	Rod	+ve	- ve	+ve
LBL4	+ve	Rod	+ve	- ve	+ve
LBL5	+ve	Rod	+ve	- ve	+ve
LBL6	+ve	Rod	+ve	- ve	+ve
LBL7	+ve	Rod	+ve	- ve	- ve

+ve, positive; - ve, negative.

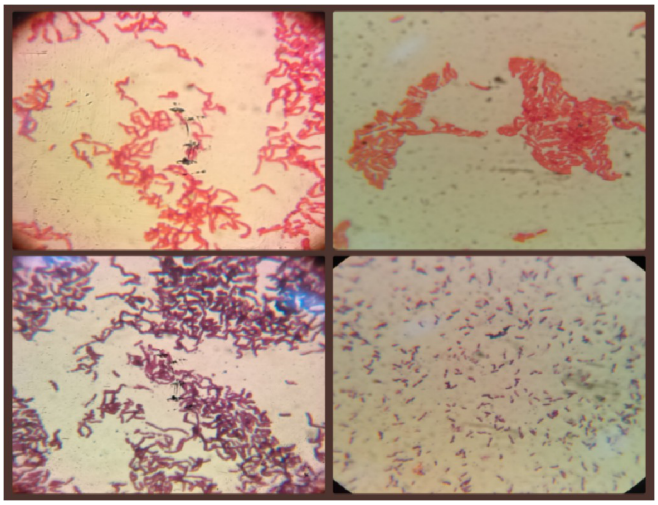

Fig. 3. Microscopic analysis of bacterial endophytes.

with 13 and 12 mm of zone of inhibition respectively. Against *Klebsiella pneumoniae* highest antimicrobial activity was shown by SBL8 with zone of clearance of 13 mm. The extracts of LBL2, SBL7 and LBL6 exhibited high antimicrobial activity (12 mm) against *Acinetobacter baumannii.* High antibacterial activity was exhibited by SBL3 against *Listeria monocytogenes* (13 mm) followed by extracts SBL12, SBL5 and SBL6 (Table 2).

3.4. Molecular characterization of endophytic bacteria

On the basis of results of antibacterial activity 3 bacterial strains (LBL 6, SBL 3 and SBL4) were selected for identification of the basis of molecular characteristics. LBL6 was identified as *Bacillus cereus* with similarity index of 96.91 % while SBL3 was identified as *Bacil-*

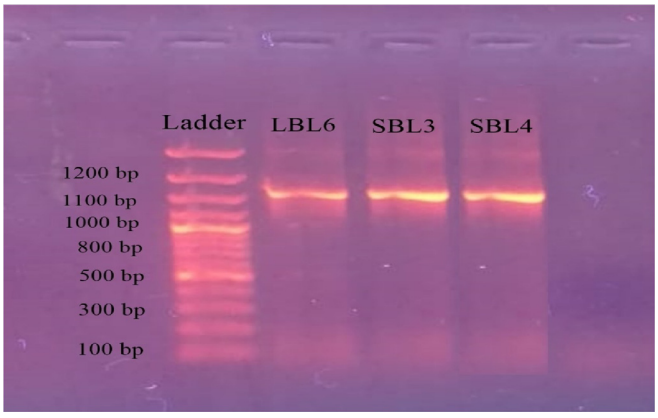

Fig. 4. 16S rRNA gene amplification of selected endophytic bacteria.

Table 2
Antibacterial activity of bacterial extracts against ATCC strains at a concentration of 1000 μg/mL.

Endophytic bacterial extracts	Antibacterial activity (Zone of clearance in mm)						
	Bs	Lm	St	Sa	Ec	Kp	Ab
SBL1	8 ± 0.5	8 ± 0.25	12 ± 0.1	11 ± 0.1	13 ± 0.2	11 ± 0.25	10 ± 0.11
SBL2	7 ± 0.1	8 ± 0.15	6 ± 0.11	5 ± 0.15	0.0 ± 0.05	10 ± 0.15	8 ± 0.1
SBL3	11 ± 0.15	13 ± 0.1	11 ± 0.5	12 ± 0.2	13 ± 0.15	10 ± 0.05	11 ± 0.15
SBL4	12 ± 0.05	10 ± 0.15	11 ± 0.15	10 ± 0.11	12 ± 0.11	10 ± 0.1	9 ± 0.25
SBL5	12 ± 0.5	11 ± 0.05	10 ± 0.05	5 ± 0.05	5 ± 0.1	10 ± 0.5	8 ± 0.05
SBL6	12 ± 0.15	11 ± 0.1	11 ± 0.25	10 ± 0.5	8 ± 0.25	10 ± 0.1	10 ± 0.1
SBL7	12 ± 0.25	9 ± 0.2	13 ± 0.05	9 ± 0.25	11 ± 0.05	12 ± 0.15	12 ± 0.11
SBL8	11 ± 0.1	8 ± 0.25	12 ± 0.1	10 ± 0.1	11 ± 0.1	13 ± 0.2	11 ± 0.2
SBL9	9 ± 0.1	10 ± 0.15	11 ± 0.11	10 ± 0.05	9 ± 0.11	10 ± 0.5	8 ± 0.15
SBL10	0.0 ± 0.05	8 ± 0.11	0.0 ± 0	0.0 ± 0.05	0.0 ± 0	0.0 ± 0	0.0 ± 0
SBL11	14 ± 0.1	10 ± 0.5	11 ± 0.5	11 ± 0.2	8 ± 0.15	11 ± 0.05	11 ± 0.5
SBL12	11 ± 0.15	11 ± 0.2	11 ± 0.05	9 ± 0.5	11 ± 0.5	10 ± 0.1	10 ± 0.2
SBL13	7 ± 0.1	8 ± 0.5	12 ± 0.11	5 ± 0.2	0.0 ± 0	0.0 ± 0.05	11 ± 0.05
LBL1	14 ± 0.11	5 ± 0.1	10 ± 0.15	10 ± 0.05	9 ± 0.11	12 ± 0.2	5 ± 0.2
LBL2	13 ± 0.05	10 ± 0.05	14 ± 0.1	10 ± 0.5	11 ± 0.05	10 ± 0.25	12 ± 0.11
LBL3	13 ± 0.1	8 ± 0.1	9 ± 0.2	10 ± 0.2	7 ± 0.2	10 ± 0.1	7 ± 0.5
LBL4	12 ± 0.1	9 ± 0.2	13 ± 0.05	10 ± 0.11	8 ± 0.1	12 ± 0.5	9 ± 0.15
LBL5	13 ± 0.05	0.0 ± 0.05	11 ± 0.15	7 ± 0.05	11 ± 0.5	11 ± 0.05	0.0 ± 0
LBL6	19 ± 0.1	10 ± 0.15	14 ± 0.2	13 ± 0.1	11 ± 0.25	12 ± 0.2	12 ± 0.05
LBL7	13 ± 0.5	10 ± 0.05	13 ± 0.2	0.0 ± 0	11 ± 0.11	8 ± 0.11	9 ± 0.1
CAM	20 ± 1.5	21 ± 1.5	25 ± 1.4	25 ± 1	20 ± 1	22 ± 1.4	19 ± 1.4

Bs: Bacillus spizizenii, Lm: Listeria monocytogenes, St: Salmonella typhimurium, Sa: Staphylococcus aureus, Ec: Escherichia coli, Kp: Klebsiella pneumoniae, Ab: Acinetobacter baumannii, CAM: Chloramphenicol.

Table 3
Molecular characterization of selected endophytic bacterial strains.

Source	Isolates	16 s rRNA Gene Length	% Similarity	Accession no. of Similar Strains
Leaf	LBL_6	1178 bp	96.91% with *Bacillus cereus*	KY750691.1
Stem	SBL3	1190 bp	97.08% with *Bacillus thuringiensis*	JX885491.1
Stem	SBL_4	1190 bp	95.77% with *Bacillus anthracis*	KP813855.1

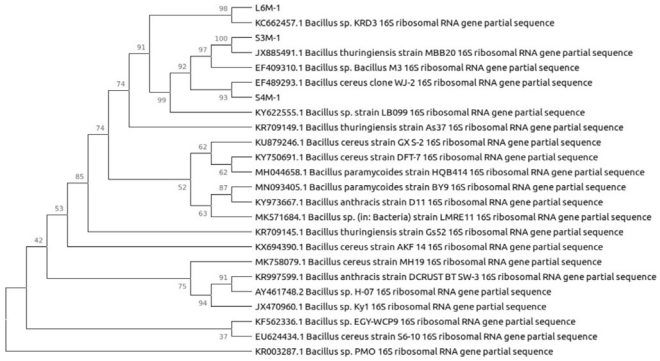

Fig. 5. A neighbor joining tree: Depicting the phylogenetic relationship of LBL6, SBL3 and SBL4. The tree has been constructed using (16 sRNA region) MEGA X. Bootstrap values are shown at respective node.

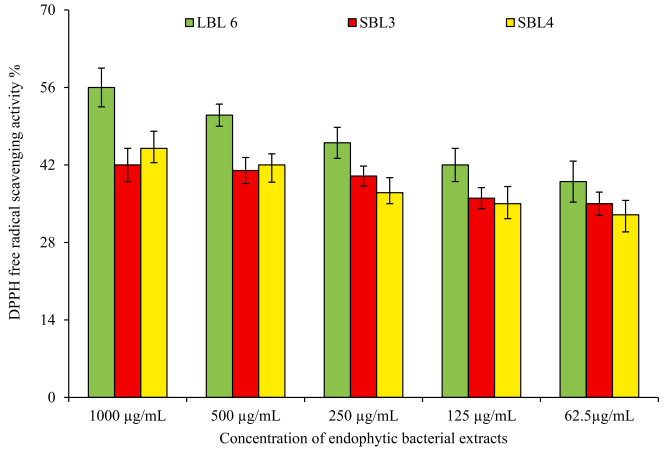

Fig. 6. Antioxidant activities of bacterial endophytes.

lus thuringiensis and SBL4 was identified as *Bacillus anthracis* by similarity index of 97.08 and 95.77 % respectively (Table 3 & Fig. 5)

3.5. DPPH free radical scavenging assay

Different concentrations of crude ethyl acetate extract of LBL6 (*Bacillus cereus*), SBL3 (*Bacillus thuringiensis*) and SBL4 (*Bacillus anthracis*) were used to determine antioxidant activity. Dose dependent antibacterial effect of bacterial extracts was recorded. LBL6 exhibited best antioxidant activity of 56 % at 1000 μg/mL concentration followed by SBL 4 and SBL 3 that is 45 and 42 % respectively (Fig. 6). LBL 6 indicated best antioxidant potential with IC_{50} value at a concentration of 574.13 μg/mL.

3.6. Antifungal activity

Antifungal activity of crude ethyl extracts was determined against two pathogenic fungal strains *A. niger* and *A. flavus*. Against *A. niger* best activity (60 % inhibition of fungal growth) was exhibited by SBL3 inhibition of fungal growth followed by LBL6 and SBL4

that is 57 and 47 % of growth inhibition. *A. flavus* was found to be most susceptible to ethyl acetate extract of SBL4 followed by SBL3 and LBL 6 respectively that is 56, 47 and 43 % of fungal growth inhibition respectively (Fig. 7).

3.7. GC–MS analysis of LBL6

Crude extract of endophytic bacterial strain LBL6 was analyzed by GC–MS analysis. Presence of 12 compounds were identified by GC–MS analysis namely Piperidinone, N-(4-Bromo-N-Butyl)- Phytol, Tricosanal , Octadecanal, 2-bromo- β-sitosterol acetate, Cholest 5-en-3-ol- 3 beta acetate, Benzoic acid 2-(1-oxopropyl)-, methyl ester, Phthalic acid, methyl 2-nitro phenyl ester, 1,2-benzenedicarboxylic acid dimethyl ester, Phthalic acid,methyl phenyl ester and 1,2-benzenedicarboxylic acid and methyl phenyl ester (Table 4, Fig. 8 & Fig. 9.).

4. Discussion

Endophytic bacteria are common inhabitants of plant tissues. Medicinal plants harbor plethora of endophytic microbes capable of producing secondary metabolites with medicinal properties

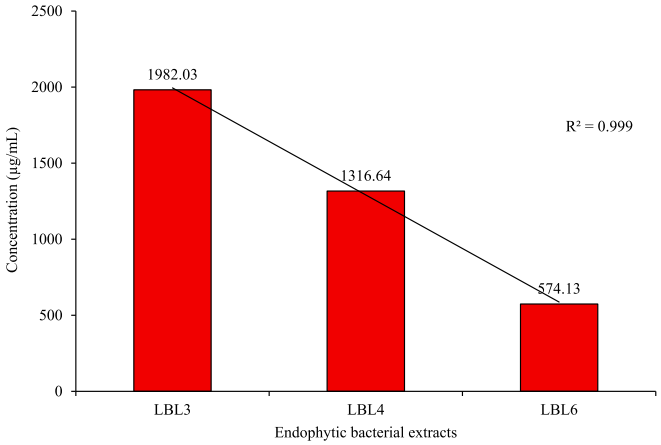

Fig. 7. Comparison of free radical scavenging potential of endophytic bacterial extracts in terms of IC_{50} by DPPH, assay. n = 3, $p = \leq 0.05$.

(Strobel and Daisy, 2003). Diversity of bacterial endophytes within the plant tissues depends on various factors like soil and environmental conditions, plant species and tissue type (Adams and Kloepper, 2002). Present study resulted in isolation of 20 strains of bacterial endophytes from *Berberis lycium*, 13 from stem and 7 strains from the leaf explants. Present results revealed that population of endophytic bacteria were higher in stem as compared to leaves. These results are in agreement with the previously reported findings of Altalhi (2009) and Rafat et al. (2012) who also reported more population of bacterial endophytes in stem as compared to leaves of grapevine and *Centella asiatica* respectively.

Results of molecular analysis of endophytic bacterial strains indicated that they are homologue to *Bacillus cereus* (LBL6), *Bacillus thuringiensis* (SBL3) and *Bacillus anthracis* (SBL4). *Bacillus cereus* was isolated from leaf while *Bacillus thuringiensis* and *Bacillus anthracis* were isolated from stem of *Berberis lycium*. All identified bacteria are Gram positive and rod-shaped indicating dominance of Gram-positive bacteria within soil and hence plants as well. Similar results had been previously reported from stem and leaves of Pistacia atlantica where *Bacillus anthracis, Bacillus pumilus* and *Pseudomonas protegens* were isolated as endophytes (Etminani and Harighi, 2018). Similarly, *Bacillus cereus, Bacillus thuringiensis* and *Bacillus subtilis* were isolated from the root nodules of *Cicer arietinum* (Egamberdieva et al 2017).

Crude extracts of bacterial endophytes exhibited varied levels of antibacterial activity against selected pathogenic strains justifying their bioactive potential. These results are in agreement with previously published report where endophytic bacteria Bacillus cereus and P. putida isolated from *Curcuma longa* showed significant antibacterial activity against *Escherichia coli* (Kumar et al. 2016). Endophytic bacteria isolated from *Murraya koenigii* also exhibited significant antibacterial activity against *Escherichia coli* (Kumar et al. 2015). Similarly in an effort to isolate and evaluate bioactive potential of bacterial endophytes from a traditional Chinese herbal plant, Li et al. (2018) reported *Bacillus* genus most dominant genus with high antimicrobial activity against tested pathogenic strains of bacteria and fungi. Akinsanya et al. (2020) while working on endophytic bacteria from *Aloe vera* plant reported that *Bacillus* species can produce several lipopeptides biosurfactants and antibiotics with ability to inhibit bacterial and fungal growth. Bioactive compounds produced by *Bacillus* strains were diverse in nature with wide range of bioactivities. Similarly Bacillus cereus and Bacillus subtilis has been isolated from *Oreochromis mossambicus*

Table 4
GCMS analysis of ethyl acetate extract of endophytic bacteria (LBL6).

S. No	Compound Name	Retention time	Mol. Weight	Formula	Biological Activity
1	2-Piperidinone,N-(4-Bromo-N-Butyl)-	3.05	233	$C_9H_{16}ONBr$	Antimicrobial activities are reported. Also reported as pesticide (Al-Bahadily et al. 2019)
2	Phytol	3.05	296	$C_{20}H_{40}O$	Antioxidant activities are reported (Santos et al. 2013)
3	Tricosanal	3.80	338	$C_{23}H_{46}O$	Antimicrobial activity is reported (Barowska et al. 2017)
4	Octadecanal, 2-bromo-	3.80	346	$C_{18}H_{35}OBr$	Antibacterial, antifungal and antimicrobial activities are reported(Kadhim et al. 2016)
5	Eicosanal	3.80	296	$C_{20}H_{40}O$	Antifungal and antimicrobial activities are reported (Mohamad et al. 2018)
6	β-sitosterol acetate	4.58	456	$C_{31}H_{52}O_2$	Antibacterial activities are reported (Ododo et al. 2016)
7	Cholest 5-en-3-ol- 3-beta acetate	4.58	428	$C_{29}H_{48}O_2$	Antimicrobial activities are reported (Bhardwaj, 2018)
8	Benzoic acid, 2-(1-oxopropyl)-, methyl ester	12.88	192	$C_{11}H_{12}O_3$	Antimicrobial, antifungal and antioxidant activities are reported (Vimalavady and Kadavul, 2013)
9	Phthalic acid, methyl 2-nitro phenyl ester	12.88	301	$C_{15}H_{11}O_6N$	Anti-inflammatory activity is reported (Krishnamoorthy and Subramaniam, 2014)
10	1,2-Benzene di carboxylic acid dimethyl ester	13.20	194	$C_{10}H_{10}O_4$	Anticancer activity is reported(Gennas et al. 2009)
11	Phthalic acid, methyl phenyl ester (1,2-Benzenedicarboxylic acid, methyl phenyl ester)	13.20	256	$C_{15}H_{12}O_4$	Antibacterial activity has been reported (Idan et al., 2015)
12	Dimethyl phthalate	12.97	194	$C_{10}H_{10}O_4$	Antifungal and antimicrobial activities are reported (Zhang et al. 2018a,b)

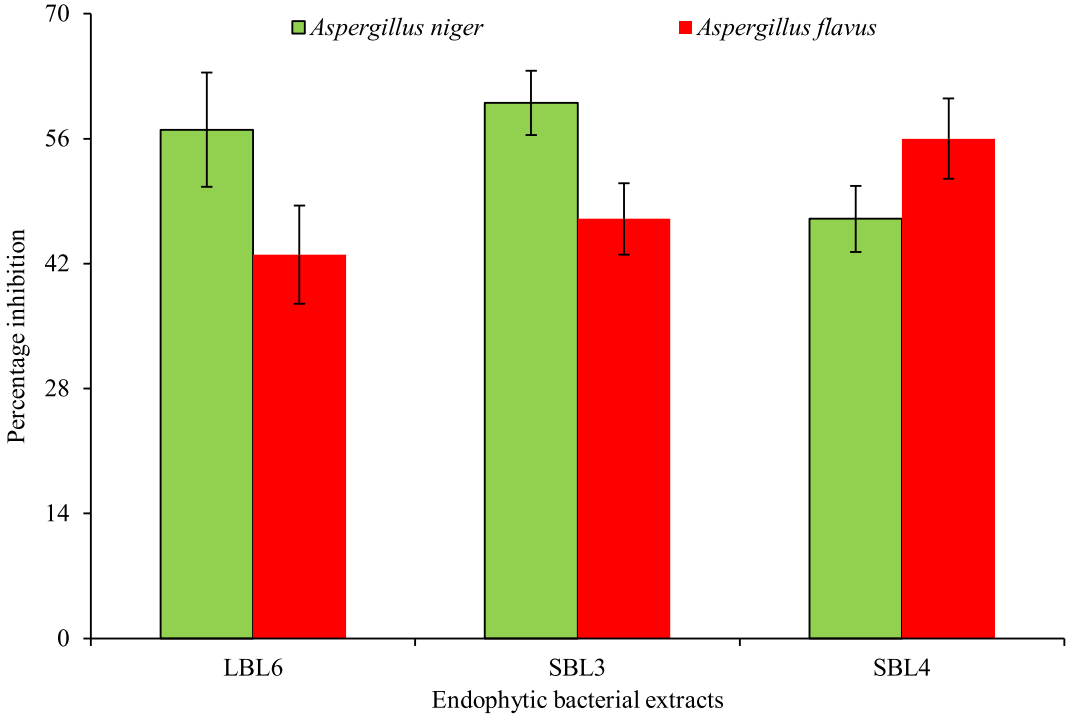

Fig. 8. Antifungal activities of endophytic bacterial extracts.

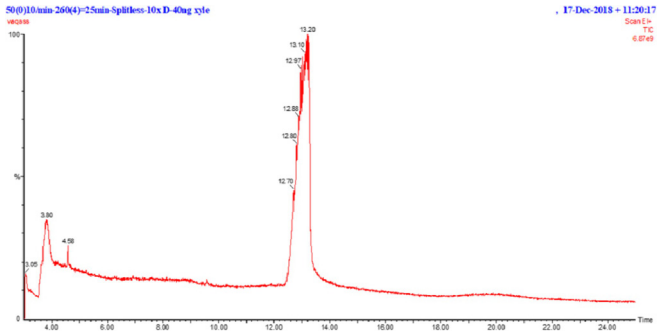

Fig. 9. GC–MS Spectrum of ethyl acetate extract of LBL6 indicating peaks for elution of bioactive compounds with respect to retention time.

and *Labeo rohita* with strong antibacterial activity against both Gram positive and negative bacteria. Compounds produced by these strains also exhibited anticancer activity. According to previous studies *Bacillus* strains retain their potential to produce bioactive compounds when they continue to grow in challenging environment like bodies of plants, animals and nematods etc that could be due to mutualistic or antagonistic associations required for their survival (Kumar et al. 2014; Seerangaraj et al. 2017)

Results of DPPH free radical scavenging assay indicated moderate to low antioxidant activity of LBL6 followed by other identified strains at different concentrations. Previously Akinsanya et al. (2015) has reported the isolation and antioxidant activities of *Bacillus* strains namely Bacillus *cereus, Bacillus thuringiensis,* Bacillus aerophilus, Bacillus niacini, Bacillus anthracis, and Bacillus stratosphericus from *Aloe vera*. In agreement to our findings Srinivasan et al. (2010) reported strong antioxidant potential of endophytic bacteria Phyllosticta and *Bacillus sp*. isolated from the Guazuma tomentosa. Antioxidant activity of extracts can be associated with presence of bioactive metabolites.

All isolated strains of endophytic bacteria exhibited moderate to low inhibition of mycelial growth (60–43%). Similar to our finding, *Bacillus amyloliquefaciens* and *Bacillus subtilis* significantly inhibited growth of blossom blight causing fungus (*Botrytis cinerea)*. In another study genomic screening of these bacterial endophytes revealed the presence of genes for synthesis of antifungal peptides (Nakkeeran et al. 2020). In agreement to our finding, isolation of *Bacillus* sps. from medicinal plants with significant antifungal activity has been reported (Ebrahimi et al. 2010, Jiang et al. 2015; Gao et al. 2017).

GCMS analysis of ethyl acetate extract of *Bacillus cereus* indicated presence of 12 compounds including 2-Piperidinone,N-(4-Bromo-N-Butyl)-, Phytol, Tricosanal, Octadecanal, 2-bromo-, Eicosanal, β-sitosterol acetate, Cholest 5-en-3-ol- 3-beta acetate, Benzoic acid, 2-(1-oxopropyl)-, methyl ester, Phthalic acid, methyl phenyl ester (1,2-Benzenedicarboxylic acid, methyl phenyl ester) and Dimethyl phthalate. Presence of these compounds can be linked with the bioactive potential of bacterial extract as previously these compounds were reported for antibacterial, anti-fungal, antioxidant, anticancer and anti-inflammatory activities (Bhardwaj, 2018; Krishnamoorthy and Subramaniam, 2014, Vimalavady and Kadavul, 2013, Idan et al. 2015). Based on the results of bioassays and GCMS analysis of extract it is clear that bioactive compounds are present in bacterial extracts. In future these compounds can be purified and used to develop antimicrobial drugs.

5. Conclusion

According to our findings diversity of endophytic bacteria is present in stem and leaves of *Berberis lycium*. These strains have ability to produce secondary metabolites with significant pharmaceutical potential. More efforts are required on purification of bioactive compounds from these extracts and to determine their mechanism of action and selective toxicity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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