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Estimation of bioactive potential of culturable bacterial endophytes from *Coleus*

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Endophytic microflora is source of several bioactive compounds. Endophytes isolated from *Coleus* species are yet to be fully explored for their bioactive potential. In this study, bacterial endophytes were isolated from three different species of *Coleus.* Isolated endophytes were characterized by using Gram staining and by sequencing 16S rRNA region. Further, solvents with different polarities were used to prepare extracts which were used for assessment of different bio-activities including *in vitro* cytotoxicity, anti-microbial and anti-oxidant activities. Also, the pure endophytic bacterial cultures were evaluated for their antiphytopathogen potential as well as indole-3-acetic acid (IAA) and protease production. Advanced studies on the endophytes with promising activities may lead to the isolation of novel natural products for drugs as well as in industrial and agricultural applications.

Keywords: Antimicrobial, Antioxidant, Antiphytopathogen, Cytotoxicity, Growth promotion, Protease production

Natural products have played a vital part in human life and are utilized as a source of medicine due to their low cost and availability. In the past century, microbes have emerged as key sources of novel natural compounds with remarkable bioactive potential. Microbes have found use as drugs as well as in industrial and agricultural applications[1]. As compared to plants, microorganisms are known to produce a larger diversity of bioactive natural products. Microorganisms that reside in plant tissues are known as endophytes[2]. Endophytes have a symbiotic relationship with their host plants and acquire several properties that are beneficial for the fitness of the host[3]. Also, several natural compounds have been identified from endophytes[4]. Further, for their survival inside the host, endophytes may activate certain biosynthetic gene clusters (BGC) and become metabolically more active as compared to their other forms[5].

Plant species belonging to the genus *Coleus* (Lamiaceae) are well known for their biological activities and thus, have been used as traditional medicine. Natural products including essential oils, diterpenoids, flavonoids, glycosides, phenolics, *etc.* isolated from *Coleus* species were found to have a range of biological activities such as antioxidant, antimicrobial, anti-urolithiatic, anti-dyspeptic, anti-inflammatory, anti-obesity activities, *etc*[6,7]. Hydro-alcoholic extract of *Coleus aromaticus* was shown to have potent antioxidant activity, anti-clastogenic activity and radio-protective activity on gamma radiation-exposed fibroblast cells (V79) of hamster[8]. Aqueous extract of *Coleus aromaticus* was reported to have free radical scavenging activity[9]. Antioxidant activity was also reported in the plant extracts of other species of *Coleus* such as *Coleus blumei*, *Coleus forskohlii* and *Coleus aromaticus*[7,10]. Roots of *C. forskohlii* accumulate medicinally valuable compounds and are used in ayurvedic medicines to treat diseases related to the heart, lungs, hypothyroidism, anti-obesity, glaucoma and several other functions in the body[11].

The microbial resources present in medicinal plants belonging to the genus *Coleus* are yet to be fully explored and very little information is available about their bioactive potential. Earlier, endophytes were reported from *C. forskohlii* and *C. aromaticus*[12,13]. In the present study, an assessment of the bioactive potential of new culturable bacterial endophytes was carried out from different species of *Coleus i.e.*, *Coleus forskohlii* (Willd.) Briq., *Coleus blumei* Benth. and *Coleus aromaticus* Benth., which may eventually lead to drug discovery as well as products with potential industrial applications.

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Materials and Methods

Collection site of plant material

The plant material of *C. forskohlii*, *C. blumei* and *C. aromaticus* were collected from the experimental fields of CSIR-IIIM (Jammu, India) during the month of October. The plants were vegetatively propagated using cuttings. The cuttings were planted on the ridges with spacing of 60-65 cm between plants. The ridges were separated with furrows of 30-40 cm. The roots, leaf and stem of all three *Coleus* species were used for the isolation of bacterial endophytes..

Isolation of bacterial endophytes

The endophytes were isolated from different tissues of three different species of *Coleus*. The fresh plant material was surface sterilized by washing with autoclaved distilled water followed by 70% ethanol and 2% sodium hypochlorite. The final step of surface sterilization included multiple washes with autoclaved distilled water and the effectiveness of surface sterilization was confirmed as described earlier[12]. Tryptone soya agar (TSA) plates were used for the isolation of bacterial endophytes from surface-sterilized plant material. The plates were incubated at 37°C for 4 d for isolation of bacterial cultures. The cultures were purified through sub-culturing several times. The pure endophytic cultures were deposited and preserved in Sir RN Chopra Microbial repository, CSIR-IIIM, Jammu (WDCM reg. no. 1117).

Microscopy and molecular characterization of isolates

The isolated bacterial endophytes were characterized based on their morphological attributes such as color and growth pattern on the plates. Microscopic characterization was done by using Gram staining.

For molecular identification and characterization, the genomic DNA was isolated from 5 mL of bacterial culture, using ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, USA). The Quality and quantity of isolated genomic DNA were assessed. Using isolated genomic DNA as a template, the variable region of the 16S rRNA region was amplified. The sequence of 16S rRNA primers is given in (Table 1). The amplicons were checked on agarose gel (1% *w/v*) and purified using Qiaex® II gel extraction kit (Qiagen, Germany). The samples were sequenced through Sanger sequencing. The sequences were analyzed using NCBI BLAST and submitted to NCBI Genbank[14].

Bioactive evaluation

Extracts Preparation

Isolated bacterial endophytes were grown in Tryptic Soy Broth (TSB) and kept at 37°C for 5 d at 180 rpm in an incubator shaker (New Brunswick, USA). The cell pellet and the broth were separated. Then, the broth was extracted with two solvents, first with ethyl acetate followed by butanol whereas the cell pellet was extracted once with methanol. The extracts were prepared by drying the solvents using rotary evaporator. These extracts were used for the estimation of the bioactive potential of these isolates.

Anti-microbial activity

Crude extracts (10 mg/mL in methanol) were used for preliminary screening of isolates for their antimicrobial potential using the agar-well diffusion method[15]. A panel of reference microorganisms was used for estimation of antimicrobial potential of endophytes; gram-negative strains: *Escherichia coli* (MTCC 730), *Klebsiella pneumonia* (ATCC 75388) and *Streptococcus pyogenes* (MTCC 442); gram-positive strains: *Bacillus cereus* (IIIM 25), *Bacillus subtilis* (MTCC 121), *Micrococcus luteus* (MTCC 2470), *Staphylococcus aureus* (MTCC 96); and the fungal strains *Candida albicans* (ATCC 90028) and *Saccharomyces cerevisiae* (MRCJ 92). The panel of microbes against which anti-microbial activity was done, were grown in broth for 24 h. 100 µL of cultures were spread on the plates using the sterilized spreader. 40 µL of endophytic extract (10 mg/mL in methanol) was filled in the wells which were prepared by scooping out the media using sterilized cork borer or sterilized tips. After the incubation of 24 h, the inhibition zones were recorded.

Cytotoxicity assay

The cytotoxicity of extracts of isolated endophytes was tested using a colorimetric assay to quantify the proliferation and the cell survival[16]. The stock solutions of extracts (10 mg/mL) were prepared in Dimethyl Sulfoxide (DMSO) and were used for the assessment of the anti-cancer potential of the isolated endophytes. Three human cancer cell lines: A549 (Lung), HCT-116 (Colon), and MCF-7 (Breast) were

Table 1 — Primer sequence of 16s rRNA used for molecular identification

Primer	Primer code	Sequence	*T*m (°C)
16s rRNA forward primer	27F	5′-AGAGTTTGAT CMTGGCTCAG-3′	55
16s rRNA reverse primer	1525R	5′-AAGGAGGT GWTCCARCC-3′	55

procured from U.S. National Cancer Institute (NCI). These cell lines were grown and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin. The culture was incubated at 37°C, 5% CO_2 and 98% relative humidity (RH) in the incubator (New Brunswick, USA). 96 well flat-bottom plates were used in this assay. Cell suspension of suitable cell seeding density was added to the plates and incubated for 24 h under culture conditions. Post-incubation, cells were treated with complete growth medium containing 100 µg of endophytic extracts. Paclitaxel, 5-Fluorouracil and Doxorubicin were used as controls. The plates were incubated again at 37 °C for 48 h. After incubation, the cells were fixed with trichloroacetic acid (TCA) at 4°C for 1 h and plates were rinsed thrice with water followed by air drying. Subsequently, 100 µL of 0.4% sulforhodamine B (SRB) dye was added and the plates were incubated at room temperature (22-28°C) for 30 min. To remove the unbounded SRB dye, the plates were washed thrice with 1% (*v/v*) acetic acid and the plates were dried at room temperature. 100 µL of TRIS buffer (pH-10.4) was added to each well and the plates were kept on the shaker for 5 min which was solubilized the protein bounded SRB dye. Optical density was measured at 540 nm. Cell growth inhibition was determined as described earlier [12]

Antioxidant activity

The antioxidant potential of endophytic extract was evaluated using the method earlier described[16]. Radical scavenging activity is based on the principle that 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical accepts a hydrogen (H) atom from the scavenger molecule known as antioxidant that reduces DPPH to form diphenylpicryl hydrazine, which is yellow colored and results in the decrease in absorbance at 517 nm. Ascorbic acid was used as positive control and percent inhibition was determined as described earlier[12,17].

Anti-phytopathogen activity

The anti-phytopathogen activity of endophytic cultures was carried out against *Verticillium dahlia* (MRCJ 198) and *Fusarium oxysporum* (MTCC 1755)[18,19]. The YEM plates were prepared and divided into two parts using a marker. On one half of the plate, the pathogen was inoculated and on the other half, the test sample was streaked. The plates with pathogen only were treated as control plates. The plates were incubated at 28°C for 4-7 d. The inhibition of pathogen growth was examined and the results were compared with the control plate.

Indole-3-acetic acid (IAA) production

Indole-3-acetic acid (IAA) production by the isolated bacterial endophytes was screened. Endophytes were grown in Nutrient Broth medium supplemented with 0.2% of L-tryptophan and incubated at 30°C on the shaker at 120 rpm for 48 h. Thereafter the broth was centrifuged at 8000 rpm for 30 min. The supernatant was mixed with Salkowski reagent (mixture of 35% perchloric acid and 0.5 M ferric chloride) in 2:1 ratio and incubated for 1 h. the absorbance was measured at 530 nm. The IAA production was determined by plotting the standard curve of IAA after measuring the optical density (OD) at 530 nm using a spectrophotometer[20].

Protease production

For screening of protease-producing endophytes, the cultures were stabbed at the center of skim milk agar plates (0.1% peptone, 0.5% NaCl, 10% skim milk, and 2% agar) and incubated at 30°C for 4-5 d. The clear zone that appeared around the colony indicated the ability of the endophyte to produce proteases. The diameter of the colony and the clear zone was measured, and the difference between the two diameters indicated the efficiency of protease activity[21].

Results and Discussion

Endophytic bacteria isolated from medicinal plants have the capability of producing various metabolites with pharmaceutical and industrial importance[5]. The diversity of endophytes depends upon various factors such as environmental conditions, host species, host developmental stage, plant organ used for endophyte isolation, *etc*[22]. Endophytes are beneficial for host health and help the host to survive in a particular environment[3]. In the present study, bacterial endophytes were isolated and identified from the root, stem, and leaf of three different species of *Coleus i.e*., *Coleus aromaticus* Benth., *Coleus blumei* Benth. and *Coleus forskohlii* (Willd.) Briq. and their bioactive potential was assessed. The plants were selected based on their medicinal properties and also for the reason that endophytic microflora of these species has not been studied in detail, especially concerning their bioactivities. Endophytes isolated from the medicinal plants displayed diverse taxonomic positions and are

known to produce various secondary metabolites with pharmaceutical and industrial importance[5]. A total of thirty bacterial endophytes were isolated and morphologically identified based on their color, shape of colonies, and growth pattern, followed by microscopic characterization (Fig. 1 and Table 2). Bacterial endophytes were differentiated between two large groups of bacteria Gram-positive and Gram-negative using Gram staining based on their cell wall constituents. All prokaryotic organisms consist of conserved and varied sequences of 16S rRNA. The bacterial species were identified by analyzing the sequence of variable region amplified using universal primers that bind to the conserved flanking sequences[23]. The sequence similarity percentage was determined using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search against 16S rRNA sequences available in NCBI GenBank (https://www.ncbi.nlm.nih.gov/). The sequences that exhibit ≥ 99% identity belong to the same species whereas the same genus exhibits ≥ 97% identity in the 16S rRNA sequence[24]. The bacterial endophytes isolated from the root and leaves of *C. forskohlii* belong to the genus *Bacillus*. Endophytic bacteria isolated from *C. aromaticus* showed high sequence similarity with different species of the genus *Bacillus* except one which was isolated from the leaf of *C. aromaticus* and showed high sequence similarity with *Alcaligenes faecalis*. The endophytes isolated from *C. blumei* were showing high sequence similarity with species of *Bacillus* and one exhibited sequence similarity with *Enterobacter aerogenes*. Earlier, several members of genus *Bacillus* were isolated from medicinal plants. For instance, *B. amyloliquefaciens was* isolated from *Ophiopogon japonicus*[25]; *B. methylotrophicus* was isolated from root endosphere of apple tree[26]; *B. subtilis*, *B. pumilus*, *B. licheniformis*, *B. cereus,* and *B. amyloliquefaciens* were isolated from maize[27]. *Alcaligenes faecalis* was reported as endophytic bacteria isolated from the roots of *Coleus forskohlii*[13] and the fruit of *Withania somnifera*[28]. *Enterobacter aerogenes* was reported as endophytic bacteria isolated from *Zea mays*[29].

Plant-microbe interaction may lead to the production of several metabolites with pharmaceutical potential[2]. Thus, preliminary screening was done for determining the antimicrobial activity of bacterial isolates against a panel of nine microbes described earlier[12]. The diameters of the inhibition zone were recorded for endophytic extracts that showed promising activity are given in (Table 3). The results indicated that ethylacetate extract of *Paenibacillus*

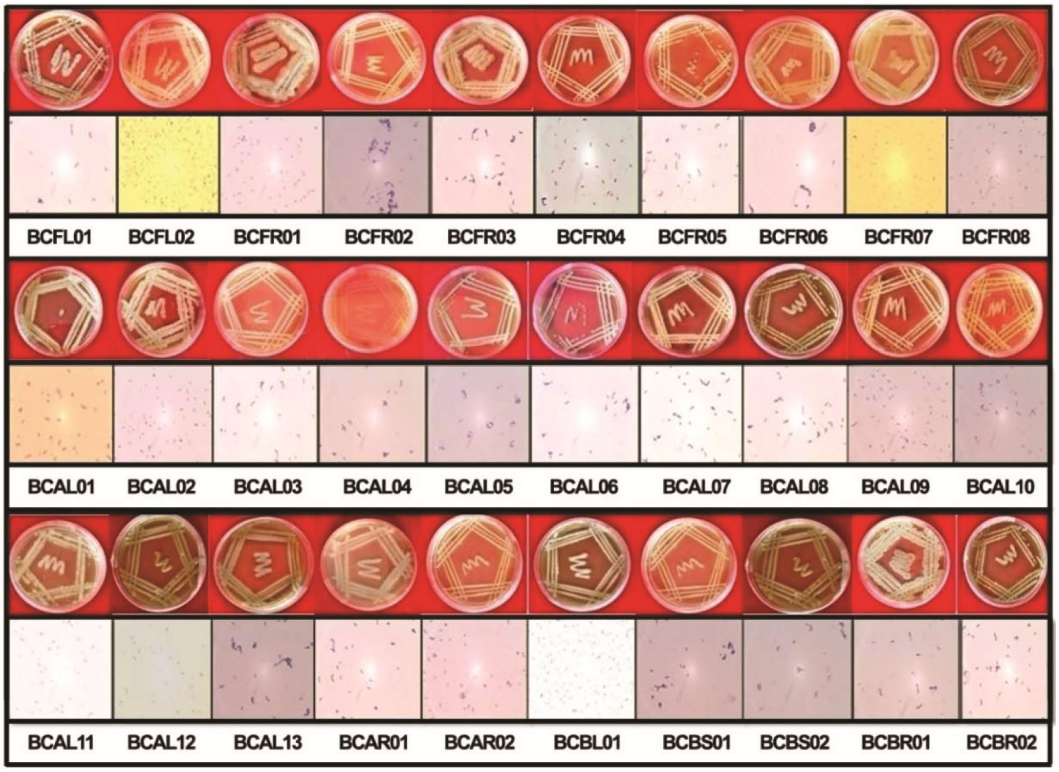

Fig. 1 — Morphology and Gram-staining of bacterial endophytes

Table 2 — Molecular identification of bacterial endophytes

Code	Plant species	Homologue species
BCFL01	*C. forskholii*	*Bacillus subtilis*
BCFL02	*C. forskholii*	*Lysinibacillus sphaericus*
BCFR01	*C. forskholii*	*Bacillus licheniformis*
BCFR02	*C. forskholii*	*Bacillus endophyticus*
BCFR03	*C. forskholii*	*Bacillus subtilis*
BCFR04	*C. forskholii*	*Bacillus megaterium*
BCFR05	*C. forskholii*	*Bacillus aryabhattai*
BCFR06	*C. forskholii*	*Bacillus subtilis*
BCFR07	*C. forskholii*	*Bacillus amyloliquefaciens*
BCFR08	*C. forskholii*	*Bacillus cereus*
BCAL01	*C. aromaticus*	*Bacillus mycoides*
BCAL02	*C. aromaticus*	*Bacillus cereus*
BCAL03	*C. aromaticus*	*Bacillus subtilis*
BCAL04	*C. aromaticus*	*Bacillus marisflavi*
BCAL05	*C. aromaticus*	*Bacillus aryabhattai*
BCAL06	*C. aromaticus*	*Bacillus megaterium*
BCAL07	*C. aromaticus*	*Bacillus ginsengisoli*
BCAL08	*C. aromaticus*	*Bacillus subtilis*
BCAL09	*C. aromaticus*	*Bacillus cereus*
BCAL10	*C. aromaticus*	*Paenibacillus alvei*
BCAL11	*C. aromaticus*	*Bacillus cereus*
BCAL12	*C. aromaticus*	*Alcaligenes faecalis*
BCAL13	*C. aromaticus*	*Bacillus safensis*
BCAR01	*C. aromaticus*	*Enterobacter bugandensis*
BCAR02	*C. aromaticus*	*Bacillus cereus*
BCBL01	*C. blumei*	*Enterobacter aerogenes*
BCBS01	*C. blumei*	*Bacillus safensis*
BCBS02	*C. blumei*	*Bacillus velezensis*
BCBR01	*C. blumei*	*Bacillus methylotrophicus*
BCBR02	*C. blumei*	*Bacillus megaterium*

Table 3 — Antimicrobial activity of endophytic extracts

Sample code	Inhibition zone (diameter of zone in mm)								
	ML	SP	BC	CA	SA	KP	EC	SC	BS
BCAL10-E	-	-	-	-	6	-	-	-	15
BCBR01-E	-	-	-	-	20	-	-	-	-
BCFL01-B	16	-	-	-	-	-	-	-	-
BCFR01-B	14	-	-	-	-	-	-	-	-
BCAL10-B	8	12	13	-	16	-	-	7	8

ML- *Micrococcus luteus,* SP- *Streptococcus pyogenes,* BC- *Bacillus cereus*, CA- *Candida albicans,* SA- *Staphylococcus aureus,* KP- *Klebsiella pneumonia,* EC- *Escherichia coli,* SC- *Sacchromyces cerevisiae,* BS- *Bacillus subtilis* E after sample code denotes the ethylacetate extract, M for methanolic extracts and B for butanolic extracts

alvei (BCAL10) showed activity against *Staphylococcus aureus* and *Bacillus subtilis* with an inhibition zone of 6 and 15 mm respectively. Ethylacetate extracts of *Bacillus methylotrophicus* (BCBR01) showed activity against *Staphylococcus aureus* with an inhibition zone of 20 mM. Butanolic extracts of *Bacillus subtilis* (BCFL01), *Bacillus licheniformis* (BCFR01) and *Paenibacillus alvei* (BCAL10) were effective against *Micrococcus luteus* with inhibition zone of 16, 14 and 8 mm respectively. Butanolic extract of *Paenibacillus alvei* (BCAL10) also showed activity against *Streptococcus pyogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Bacillus subtilis*. Earlier, *Bacillus* sp. isolated from the stem of *Phaseolus vulgaris* has been shown to possess antimicrobial activity against *B. cereus*, *Klebsiella pneumoniae* and *Citrobacter freundii*[30]. *B. amyloliquefaciens* isolated from mulberry leaves showed antimicrobial activity against *Colletotrichum dematium*[31]. Endophytic *Paenibacillus alvei* isolated from roots of *Polygonum cuspidatum* have been shown to exhibit antimicrobial activity against *Gibberella fujikuroi* and *Staphylococcus aureus*[32].

In vitro cytotoxicity assay against lung (A549), colon (HCT-116) and breast (MCF-7) cancer cell lines were carried out using extracts of isolated endophytes. 100 µg of extract (10 mg/mL in DMSO) was used for *in vitro* cytotoxicity assay. Ethylacetate and butanolic extracts were tested against different cancer cell lines (Table 4). Ethylacetate extracts of *Bacillus cereus* (BCAL02), *Bacillus safensis* (BCBS01) and butanolic extracts of *Bacillus endophyticus* (BCFR02), *Bacillus velezensis* (BCBS02) showed ≥75% cytotoxicity against lung cancer cell line (A549). Ethylacetate extracts of *Bacillus subtilis* (BCFR03), *Bacillus cereus* (BCAL02), *Bacillus subtilis* (BCAL08), *Bacillus safensis* (BCBS01) and butanolic extracts of *Bacillus endophyticus* (BCFR02), *Bacillus cereus* (BCAR02) and *Bacillus velezensis* (BCBS02) showed ≥75% cytotoxicity against colon cancer cell line (HCT-116). Ethylacetate extracts of *Bacillus subtilis* (BCFR03), *Bacillus cereus* (BCFR08), *Bacillus cereus* (BCAL02), *Bacillus aryabhattai* (BCAL05), *Bacillus subtilis* (BCAL08), *Alcaligenes faecalis* (BCAL12), *Bacillus safensis* (BCAL13), *Enterobacter aerogenes* (BCBL01), *Bacillus safensis* (BCBS01), *Bacillus velezensis* (BCBS02) and butanolic extract of *Bacillus endophyticus* (BCFR02) showed ≥75% cytotoxicity against breast cancer cell line (MCF-7). In other studies as well, the crude extract of endophyte *Bacillus safensis* isolated from the leaves of *Crinum macowanii* exhibited anticancer activity against lung Cancer[33]. *Bacillus* isolated from the stem of *Coleus aromaticus*

Table 4 — Percentage cytotoxicity of endophytic extracts

Tissue	Lung	Colon	Breast
Cell Line	A549	HCT-116	MCF-7
Sample code	Percentage Cytotoxicity (%)		
BCFL01-E	46	43	69
BCFL02-E	52	56	72
BCFR01-E#	47	45	83
BCFR02-E	24	20	45
BCFR03-E	45	77	97
BCFR04-E	13	18	45
BCFR05-E	47	27	71
BCFR06-E	53	49	73
BCFR07-E	38	29	38
BCFR08-E	31	20	75
BCAL01-E	31	28	52
BCAL02-E*	83	100	96
BCAL03-E	30	23	49
BCAL04-E	21	0	67
BCAL05-E#	34	15	86
BCAL06-E	30	4	63
BCAL07-E	7	24	54
BCAL08-E	54	100	99
BCAL09-E	0	24	46
BCAL10-E	16	37	33
BCAL11-E	41	27	56
BCAL12-E#	6	33	78
BCAL13-E#	23	14	77
BCAR01-E	22	0	68
BCAR02-E	25	13	28
BCBL01-E#	6	33	78
BCBS01-E*	79	100	99
BCBS02-E	23	14	77
BCBR01-E	36	49	69
BCBR02-E	25	30	53
BCFL01-M	23	25	10
BCFL02-M	35	13	54
BCFR01-M	37	18	48
BCFR02-M	22	0	57
BCFR03-M	34	23	66
BCFR04-M	30	20	69
BCFR05-M	26	0	30
BCFR06-M	36	0	29
BCFR07-M	17	0	51
BCFR08-M	26	30	21
BCAL01-M	36	16	63
BCAL02-M	32	6	58
BCAL03-M	35	24	35
BCAL04-M	38	7	22
BCAL05-M	25	39	72
BCAL06-M	35	8	74
BCAL07-M	28	17	52
BCAL08-M	19	10	32
BCAL09-M	22	0	36
BCAL10-M	40	0	20

(*contd.*)

Table 4 — Percentage cytotoxicity of endophytic extracts (*contd.*)

Tissue	Lung	Colon	Breast
Cell Line	A549	HCT-116	MCF-7
Sample code	Percentage Cytotoxicity (%)		
BCAL11-M	54	0	28
BCAL12-M	0	17	30
BCAL13-M	19	5	0
BCAR01-M	37	22	32
BCAR02-M	18	2	45
BCBL01-M	17	0	33
BCBS01-M	19	0	31
BCBS02-M	33	0	10
BCBR01-M	17	0	57
BCBR02-M	35	22	22
BCFL01-B	48	59	42
BCFL02-B	55	61	63
BCFR01-B	56	65	64
BCFR02-B*	99	92	100
BCFR03-B	24	69	43
BCFR04-B	30	63	38
BCFR05-B	30	48	37
BCFR06-B	36	64	61
BCFR07-B	42	24	68
BCFR08-B	12	17	18
BCAL01-B	40	61	35
BCAL02-B	34	68	63
BCAL03-B	35	72	46
BCAL04-B	18	43	32
BCAL05-B	38	56	30
BCAL06-B	46	59	33
BCAL07-B	40	70	43
BCAL08-B	24	41	37
BCAL09-B	22	58	32
BCAL10-B	27	54	37
BCAL11-B	32	58	27
BCAL12-B	0	28	41
BCAL13-B	0	30	48
BCAR01-B	17	46	31
BCAR02-B#	70	81	32
BCBL01-B	52	7	52
BCBS01-B	2	39	38
BCBS02-B	75	78	67
BCBR01-B	55	64	57
BCBR02-B	15	18	30

Concentration	Sample code	Percentage Cytotoxicity (%)		
1 µM	PACLITAXEL	72	-	-
20 µM	5-FU	-	-	52
1 µM	DOXORUBICIN	-	64	-

E after sample code denotes the ethylacetate extract, M for methanolic extracts and
B for butanolic extracts. * indicates the extracts that showed cytotoxicity against all three cancer cell lines
# indicates the extracts that showed cytotoxicity against specific cancer cell lines

showed cytotoxicity against lung, colon and breast cancer cell lines[12]. The exopolysaccharides from *Alcaligenes faecalis* have been shown to exhibit cytotoxicity against human hepatoblastoma cell line and lung cancer cell line[34]. Ethylacetate extract of *Bacillus cereus* (BCAL02), *Bacillus safensis* (BCBS01) and butanolic extract of *Bacillus endophyticus* (BCFR02) exhibited promising cytotoxicity (≥75%) against all three cancer cell lines. On the other hand, some of our extracts exhibited specific cytotoxic activity in a particular cell line, rather than a generalized cytotoxic effect on all cell lines. For instance, ethyl acetate extracts of *Bacillus licheniformis* (BCFR01), *Bacillus aryabhattai* (BCAL05), *Alcaligenes faecalis* (BCAL12), *Bacillus safensis* (BCAL13), and *Enterobacter aerogenes* (BCBL01) showed cytotoxicity specifically against human breast cancer cell lines and butanolic extract of *Bacillus cereus* (BCAR02) showed cytotoxicity specifically against colon cancer cell line and not against other two cancer cell lines.

DPPH radical scavenging ability of the extracts prepared from the isolated endophytes was assessed and IC_{50} values were calculated based on their absorbance values. The IC_{50} values of bacterial endophytes extracts are given in (Table 5). Butanolic extracts of *Bacillus cereus* (BCAL02), *Bacillus cereus* (BCAL11), *Alcaligenes faecalis* (BCAL12) and *Bacillus megaterium* (BCBR02) exhibited promising antioxidant activity with IC_{50}<10 µg. For instance, *Bacillus* isolated from the fruits of *Morinda citrifolia* showed promising antioxidant activity as well as antagonistic activity against *E. coli* and *Staphylococcus aureus*[35]. The exopolysaccharides from *Alcaligenes faecalis* showed antioxidant activity[34]. In another study, five *Bacillus* sp. isolated from the leaves of *Solenostemma argel*, *Calotropis procera* and *Hibiscus sabdariffa* showed antioxidant potential[36].

The phyto-pathogen antagonism potential of isolated endophytes was assessed by determining the inhibitory effect of isolated endophytes against plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* through dual culture assay. The percentage of inhibition was calculated by subtracting the radius of pathogen grown with tested culture from the radius of control culture of pathogen without tested culture and recorded in (Table 6). *Bacillus licheniformis* (BCFR01), *Bacillus aryabhattai* (BCFR05), *Bacillus subtilis* (BCFR06), *Bacillus cereus* (BCFR08), *Bacillus marisflavi* (BCAL04), *Bacillus megaterium* (BCAL06), *Bacillus ginsengisoli* (BCAL07), *Bacillus safensis* (BCAL13) were effective against *Verticillium dahlia* and *Bacillus licheniformis* (BCFR01), *Bacillus subtilis* (BCFR03), *Bacillus megaterium* (BCFR04), *Bacillus subtilis* (BCAL08), *Bacillus cereus* (BCAL09) and *Bacillus megaterium* (BCBR02) were effective against *Fusarium oxysporum.* Earlier studies have shown that members of genus *Bacillus* exhibited a high degree of antagonistic potential against plant pathogens[37,38]. For instance, endophytic *Bacillus subtilis* isolated from the leaves of medicinal plant *Prunus cerasifera* showed anti-phytopathogen activity against five pathogens including *Verticillium dahliae* and *Fusarium oxysporum*[37]. In another study, *Bacillus velezensis* isolated from rice has been shown antagonistic activity against *Fusarium oxysporum*[38]. Endophytic *Bacillus* sp. isolated from cotton roots showed antagonistic activity against *Verticillium dahliae*[39]. Interestingly, *Paenibacillus alvei* (BCAL10) showed antifungal activity against *S. cerevisiae* though it was not active against the phytopathogens. Similarly, the endophytic extracts that were found active against the phytopathogens (Table 6) did not show a significant antifungal activity when tested against *C. albicans* and *S. cerevisiae*. This suggests that the anti-phytopathogen activity demonstrated by the endophytes could be specific.

The growth-promoting potential of isolates was determined by assessing their ability to produce IAA. *Bacillus amyloliquefaciens* (BCFR07) isolated from *C. forskohlii*; *Bacillus cereus* (BCAL09), *Bacillus cereus* (BCAL11), *Bacillus safensis* (BCAL13) isolated from *C. aromaticus* and *Bacillus velezensis* (BCBS02) isolated from *C. blumei* produced more than 50 µg/mL of IAA (Table 7). Earlier several studies have shown that *Bacillus* sp. such as *B. amyloliquefaciens* isolated from *Hedera helix*; *B. aryabhattai*, *B. megaterium* and *B. cereus* isolated from nodules of *Vigna radiate*; *B. velezensis* isolated from tomato were capable of promoting growth by producing phytohormone (IAA)[40]. Root rot of *C. forskohlii* is a devastating disease caused by *Fusarium chlamydosporum*[41]. *Fusarium oxysporum* has also been shown to be the pathogen responsible for Fusarium wilt of *C. forskohlii*[42]. It is pertinent to note that *Bacillus cereus* (BCAL09) which exhibits good IAA production was also found effective against *Fusarium oxysporum* in our anti-phytopathogen assays and could be a good candidate for future studies focusing on biocontrol of *Fusarium* wilt in *C. forskohlii*. Earlier, studies showed endophytic bacteria isolated from *Solanum sodomaeum* and *Solanum*

Table 5 — Measurement of DPPH radical scavenging activity of endophytes

Sample code	IC^{50} values		
BCFL01-E	82.05	±	0.93
BCFL02-E	277.40	±	0.30
BCFR01-E	163.53	±	0.34
BCFR02-E	279.02	±	0.11
BCFR03-E	390.33	±	0.28
BCFR04-E	178.20	±	0.54
BCFR05-E	347.55	±	0.44
BCFR06-E	324.34	±	0.43
BCFR07-E	220.45	±	0.41
BCFR08-E	542.60	±	0.30
BCAL01-E	85.33	±	0.34
BCAL02-E	145.60	±	0.50
BCAL03-E	271.76	±	0.35
BCAL04-E	191.17	±	0.29
BCAL05-E	271.45	±	0.43
BCAL06-E	196.49	±	0.45
BCAL07-E	221.46	±	0.46
BCAL08-E	309.52	±	0.23
BCAL09-E	156.74	±	0.20
BCAL10-E	286.64	±	0.38
BCAL11-E	122.28	±	0.53
BCAL12-E	214.35	±	0.43
BCAL13-E	236.53	±	0.36
BCAR01-E	293.59	±	0.52
BCAR02-E	322.30	±	0.27
BCBL01-E	282.35	±	0.32
BCBS01-E	312.88	±	0.11
BCBS02-E	274.39	±	0.30
BCBR01-E	181.20	±	0.60
BCBR02-E	220.43	±	0.26
BCFL01-M	254.52	±	1.74
BCFL02-M	182.42	±	0.50
BCFR01-M	131.06	±	1.46
BCFR02-M	295.89	±	1.65
BCFR03-M	360.19	±	1.93
BCFR04-M	175.85	±	0.62
BCFR05-M	182.57	±	0.30
BCFR06-M	109.44	±	0.29
BCFR07-M	176.65	±	0.40
BCFR08-M	404.89	±	0.11
BCAL01-M	195.74	±	1.24
BCAL02-M	75.56	±	0.64
BCAL03-M	154.35	±	0.81
BCAL04-M	185.03	±	0.50
BCAL05-M	264.71	±	0.23
BCAL06-M	191.94	±	0.84
BCAL07-M	188.91	±	1.32
BCAL08-M	162.15	±	1.83
BCAL09-M	327.06	±	2.48
BCAL10-M	241.09	±	0.23
BCAL11-M	148.45	±	0.49
BCAL12-M	170.55	±	0.25
BCAL13-M	196.42	±	0.38
BCAR01-M	328.76	±	1.28
BCAR02-M	244.37	±	0.47
BCBL01-M	232.62	±	0.47
BCBS01-M	249.49	±	0.42
BCBS02-M	227.40	±	0.51
BCBR01-M	175.56	±	0.74
BCBR02-M	163.36	±	0.25
BCFL01-B	59.48	±	0.36
BCFL02-B	65.45	±	0.39
BCFR01-B	143.50	±	0.40
BCFR02-B	212.55	±	0.27
BCFR03-B	159.26	±	0.15
BCFR04-B	218.55	±	0.25
BCFR05-B	28.47	±	0.38
BCFR06-B	162.27	±	0.17
BCFR07-B	284.36	±	0.38
BCFR08-B	58.56	±	0.36
BCAL01-B	155.35	±	0.42
BCAL02-B	3.47	±	0.41
BCAL03-B	194.31	±	0.31
BCAL04-B	129.26	±	0.23
BCAL05-B	338.56	±	0.42
BCAL06-B	185.39	±	0.52
BCAL07-B	222.32	±	0.29
BCAL08-B	155.42	±	0.32
BCAL09-B	13.43	±	0.14
BCAL10-B	57.60	±	0.03
BCAL11-B	6.65	±	0.35
BCAL12-B	8.51	±	0.34
BCAL13-B	129.50	±	0.38
BCAR01-B	300.39	±	0.46
BCAR02-B	142.42	±	0.44
BCBL01-B	319.59	±	0.18
BCBS01-B	57.33	±	0.33
BCBS02-B	169.19	±	0.13
BCBR01-B	221.49	±	0.42
BCBR02-B	8.50	±	0.40
Ascorbic acid	25.59	±	0.07

(*contd.*)

Table 5 — Measurement of DPPH radical scavenging activity of endophytes (*contd.*)

Sample code	IC_{50} values		

IC_{50}, the concentration of extracts (µg/mL) causing 50% inhibition of DPPH radical

bonariense can be promising biocontrol agents against *Fusarium oxysporum* f. sp. *lycopersici*. In another study, *Bacillus* sp. was shown as an effective biocontrol agent against *Fusarium verticillioides* which is maize pathogenic fungus[43,44].

The ability of isolated endophytes to produce protease was assessed. *Bacillus subtilis* (BCFL01), *Bacillus licheniformis* (BCFR01), *Bacillus endophyticus* (BCFR02), *Bacillus subtilis* (BCFR03), *Bacillus megaterium* (BCFR04), *Bacillus aryabhattai* (BCFR05), *Bacillus amyloliquefaciens* (BCFR07), *Bacillus cereus* (BCFR08) from *C. forskohlii*; *Bacillus aryabhattai* (BCAL05), *Bacillus megaterium* (BCAL06), *Bacillus ginsengisoli* (BCAL07), *Bacillus subtilis* (BCAL08), *Bacillus cereus* (BCAL09), *Bacillus safensis* (BCAL13) from *C. aromaticus* and *Bacillus safensis* (BCBS01) from *C. blumei* showed potential of producing protease. *Bacillus amyloliquefaciens* (BCFR07), *Bacillus cereus* (BCFR08), *Bacillus cereus* (BCAL09) and *Bacillus safensis* (BCAL13) have diameter of clear zone ≥ 3.5 cm (Table 8). Earlier several studies have shown protease and cellulase producing ability of members of *Bacillus* genus[45]. Protease and cellulase are hydrolytic

Table 6 — Percentage inhibition of phytopathogens by endophytic cultures

Sample code	Percentage inhibition (%)	
	Fo	Vd
BCFL01	46.67	40
BCFL02	40.00	26.67
BCFR01	53.33	53.33
BCFR02	33.33	33.33
BCFR03	56.67	40
BCFR04	53.33	26.67
BCFR05	46.67	60
BCFR06	50.00	66.67
BCFR07	50.00	40
BCFR08	33.33	60
BCAL01	33.33	33.33
BCAL02	33.33	40
BCAL03	43.33	40
BCAL04	46.67	66.67
BCAL05	36.67	33.33
BCAL06	43.33	66.67
BCAL07	40.00	66.67
BCAL08	50.00	46.67
BCAL09	53.33	46.67
BCAL10	26.67	20
BCAL11	40.00	66.67
BCAL12	26.67	33.33
BCAL13	40.00	66.67
BCAR01	33.33	33.33
BCAR02	30.00	26.67
BCBL01	43.33	46.67
BCBR01	36.67	40
BCBS01	40.00	33.33
BCBS02	30.00	20
BCBR01	16.67	00
BCBR02	60.00	46.67

Fo: *Fusarium oxysporum* Vd: *Verticillium dahliae*

Table 7 — Screening of IAA producing endophytes

Sample Code	IAA production (µg/ml)		
BCFL01	6.78	±	0.17
BCFL02	5.67	±	0.10
BCFR01	44.51	±	2.32
BCFR02	36.67	±	1.71
BCFR03	23.58	±	0.30
BCFR04	27.87	±	0.04
BCFR05	46.36	±	1.31
BCFR06	47.02	±	0.84
BCFR07	54.98	±	0.27
BCFR08	28.11	±	0.77
BCAL01	17.31	±	0.49
BCAL02	23.56	±	0.87
BCAL03	8.07	±	0.34
BCAL04	34.91	±	0.23
BCAL05	42.82	±	0.29
BCAL06	9.16	±	0.13
BCAL07	11.94	±	0.57
BCAL08	21.29	±	8.20
BCAL09	51.07	±	0.39
BCAL10	10.89	±	0.31
BCAL11	50.67	±	1.43
BCAL12	8.91	±	0.32
BCAL13	63.45	±	0.66
BCAR01	37.91	±	0.74
BCAR02	12.80	±	0.68
BCBL01	30.34	±	1.13
BCBS01	10.58	±	0.49
BCBS02	50.31	±	1.75
BCBR01	13.29	±	0.73
BCBR02	20.47	±	0.98

Table 8 — Screening of protease producing endophyes

Sample code	Diameter of clear zone
BCFL01	3.3
BCFL02	-
BCFR01	3.1
BCFR02	2.6
BCFR03	2.7
BCFR04	3.3
BCFR05	3.4
BCFR06	-
BCFR07	3.7
BCFR08	3.5
BCAL01	-
BCAL02	-
BCAL03	-
BCAL04	-
BCAL05	1.9
BCAL06	3.1
BCAL07	3.6
BCAL08	2.9
BCAL09	3.2
BCAL10	-
BCAL11	-
BCAL12	-
BCAL13	3.6
BCAR01	-
BCAR02	-
BCBL01	-
BCBS01	2.5
BCBS02	-
BCBR01	-
BCBR02	-

enzymes that promote degradation of fungal cell wall[46]. Thus, the endophytes which were capable of producing these enzymes can act as a bio-control agent and may also have the potential for biotechnological application.

Conclusion

In the present study, thirty bacterial endophytes were isolated from *C. blumei*, *C. forskohlii* and *C. aromaticus*. The isolates were identified and their bioactive potential was determined. Preliminary assays were carried out for antimicrobial, anti-oxidant and *in vitro* cytotoxicity activities. These assays indicate that the crude extracts of some isolated endophytes have significant bioactive potential. The pure cultures can be used further for scaling up and isolating pure compounds. Further, some of the isolates exhibited anti-phytopathogen activity and can be used as bio-control agents. Also, some of the endophytes showed IAA and protease-producing ability and can be used as growth-promoting agents.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Newman DJ & Cragg GM, Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod*, 83 (2020) 770.

2 Strobel G, Harnessing endophytes for industrial microbiology. *Curr Opin Microbiol*, 9 (2006) 240.

3 Wu W, Chen W, Liu S, Wu J, Zhu Y, Qin L & Zhu B, Beneficial Relationships Between Endophytic Bacteria and Medicinal Plants. *Front Plant Sci*, 12 (2021) 646146.

4 Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M & der Lelie Dv, Endophytic bacteria and their potential applications. *Crit Rev Plant Sci*, 21 (2002) 583.

5 Strobel G & Daisy B, Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev*, 67 (2003) 491.

6 Katnoria JK & Nagpal AK, Analytical techniques for phytochemicals screening and bioactivities of some *Coleus* Species: A review. *J Pharm Sci*, 8 (2016) 227.

7 Rasineni GK & Reddy AR, Free radical quenching activity and polyphenols in three species of *Coleus*. *J Med Plants*, 2 (2008) 285.

8 Rao BS, Shanbhoge R, Upadhya D, Jagetia GC, Adiga SK, Kumar P, Guruprasad K & Gayathri P, Antioxidant, anticlastogenic and radioprotective effect of *Coleus aromaticus* on Chinese hamster fibroblast cells (V79) exposed to gamma radiation. *Mutagenesis*, 21 (2006) 237.

9 Kumaran A & Karunakaran RJ, Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chem*, 100 (2007) 356.

10 Zakaria Z, Aziz R, Lachimanan YL, Sreenivasan S & Rathinam X, Antioxidant activity of *Coleus blumei*, *Orthosiphon stamineus*, *Ocimum basilicum* and *Mentha arvensis* from Lamiaceae family. *Int J Nat Eng Sci*, 2 (2008) 93.

11 Kavitha C, Rajamani K & Vadivel E, *Coleus forskohlii* A comprehensive review on morphology, phytochemistry and pharmacological aspects. *J Med Plants*, 4 (2010) 278.

12 Jamwal VL, Gulfam S, Manhas RS, Qayum A, Kapoor N, Chouhan R, Singh SK, Chaubey A & Gandhi SG, Isolation,

identification and bioactive potential of bacterial endophytes from *Coleus*. *Indian J Biochem Biophys*, 56 (2019) 392.

13 Mastan A, Rane D, Dastager SG & Vivek Babu CS, Plant Probiotic Bacterial Endophyte, *Alcaligenes faecalis*, Modulates Plant Growth and Forskolin Biosynthesis in *Coleus forskohlii*. *Probiotics Antimicrob Proteins*, 12 (2020) 481.

14 Mangamuri U, Kalagatur NK & Poda S, Isolation, structure elucidation and bioactivity of secondary metabolites produced by marine derived Streptomonospora arabica VSM-25. *Indian J Biochem Biophys*, 59 (2022) 73.

15 Kumari V & Sangal A, Antimicrobial study of *Arjuna Terminalia* loaded PLGA nanoparticle. *Indian J Biochem Biophys*, 57 (2020) 291.

16 Vichai V& Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*, (2006) 1112.

17 Awasthi P, Mahajan V, Jamwal VL, Kapoor N, Rasool S, Bedi YS & Gandhi SG, Cloning and expression analysis of chalcone synthase gene from *Coleus forskohlii*. *J Genet*, 95 (2016) 647.

18 Sabu R, Soumya KR & Radhakrishnan EK, Endophytic *Nocardiopsis* sp. from *Zingiber officinale* with both antiphytopathogenic mechanisms and antibiofilm activity against clinical isolates. *3 Biotech,* 7 (2017) 1.

19 Hou Z, Yang R, Zhang C, Zhu LF, Miao F, Yang XJ & Zhou L, 2-(Substituted phenyl)-3, 4-dihydroisoquinolin-2-iums as novel antifungal lead compounds: Biological evaluation and structure-activity relationships. *Molecules*, 18 (2013) 10413.

20 Shokri D & Emtiazi G, Indole-3-acetic acid (IAA) production in symbiotic and non-symbiotic nitrogen-fixing bacteria and its optimization by Taguchi design. *Curr Microbiol*, 61 (2010) 217.

21 Rajmohan S, Dodd CE & Waites WM, Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. *J Appl Microbiol*, 93 (2002) 205.

22 Dudeja SS & Giri R, Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non legumes. *Afr J Microbiol Res,* 8 (2014) 1562.

23 Ash C, Farrow JA, Dorsch M, Stackebrandt E & Collins MD, Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int J Syst Evol Microbiol*, 41 (1991) 343.

24 Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP & Raoult D, 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol*, 38 (2000) 3623.

25 Chen YT, Yuan Q, Shan LT, Lin MA, Cheng DQ & Li CY, Antitumor activity of bacterial exopolysaccharides from the endophyte *Bacillus amyloliquefaciens* sp. isolated from *Ophiopogon japonicus*. *Oncol Lett*, 5 (2013) 1787.

26 Mehta P, Walia A, Kakkar N & Shirkot CK, Tricalcium phosphate solubilisation by new endophyte *Bacillus methylotrophicus* CKAM isolated from apple root endosphere and its plant growth-promoting activities. *Acta Physiol Plant*, 36 (2014) 2033.

27 Figueiredo JE, Gomes EA, Guimarães CT, de Paula Lana UG, Teixeira MA, Lima GV & Bressan W, Molecular analysis of endophytic bacteria from the genus *Bacillus* isolated from tropical maize (*Zea mays* L.). *Braz J Microbiol*, 40 (2009) 522.

28 Abdallah RAB, Mejdoub-Trabelsi B, Nefzi A, Jabnoun-Khiareddine H & Daami-Remadi M, Isolation of endophytic bacteria from *Withania somnifera* and assessment of their ability to suppress *Fusarium* wilt disease in tomato and to promote plant growth. *J Plant Pathol Microbiol*, 7 (2016).

29 Thanh DTN & Diep CN, Isolation, characterization and identification of endophytic bacteria in maize (*Zea mays* L.) cultivated on Acrisols of the Southeast of Vietnam. *Am J Life Sci*, 2 (2014) 224.

30 Beiranvand M, Amin M, Hashemi-Shahraki A, Romani B, Yaghoubi S & Sadeghi P, Antimicrobial activity of endophytic bacterial populations isolated from medical plants of Iran. *Iran J Microbiol*, 9 (2017) 11.

31 Yoshida S, Hiradate S, Tsukamoto T, Hatakeda K & Shirata A, Antimicrobial Activity of Culture Filtrate of *Bacillus amyloliquefaciens* RC-2 Isolated from Mulberry Leaves. *Phytopathology*, 91 (2001) 181.

32 Sun H, He Y, Xiao Q, Ye R & Tian Y, Isolation, characterization, and antimicrobial activity of endophytic bacteria from *Polygonum cuspidatum*. *Afr J Microbiol Res*, 7 (2013) 1496.

33 Sebola TE, Uche-Okereafor NC, Mekuto L, Makatini MM, Green E & Mavumengwana V, Antibacterial and Anticancer Activity and Untargeted Secondary Metabolite Profiling of Crude Bacterial Endophyte Extracts from *Crinum macowanii* Baker Leaves. *Int J Microbiol*, 2020 (2020) 8839490.

34 Aullybux AA, Puchooa D, Bahorun T, Jeewon R, Wen X & Matin P, Antioxidant and Cytotoxic Activities of Exopolysaccharides from *Alcaligenes faecalis* Species Isolated from the Marine Environment of Mauritius. *J Polym Environ*, (2021) 1.

35 Sun B, Jing R, Wang Z, Tian L, Mao F & Liu Y, Diversity and community structure of endophytic *Bacillus* with antagonistic and antioxidant activity in the fruits of Xisha Wild Noni (*Morinda citrifolia* L.). *Microb Pathog*, 158 (2021) 105065.

36 Hagaggi NSA, Phenolic Contents, Antioxidant Capacity and Antibacterial Activity of Extracts from *Bacillus* spp. Associated with The Leaves of Some Medicinal Plants. *Egypt Acad J Biol Sci G Microbiol*, 12 (2020) 55.

37 Pei D, Zhang Q, Zhu X & Han S, Endophytic *Bacillus subtilis* P10 from Prunus cerasifera as a biocontrol agent against tomato *Verticillium* wilt. *Braz J Bio*, 83 (2021) e244261.

38 Cao Y, Ding W & Liu C, Unraveling the Metabolite Signature of Endophytic *Bacillus velezensis* Strain Showing Defense Response Towards *Fusarium oxysporum*. *Agronomy*, 11 (2021) 683.

39 Hasan N, Farzand A, Heng Z, Khan IU, Moosa A, Zubair M, Na Y, Ying S & Canming T, Antagonistic Potential of Novel Endophytic *Bacillus* Strains and Mediation of Plant Defense against *Verticillium* Wilt in Upland Cotton. *Plants*, 9 (2020).

40 Bhutani N, Maheshwari R, Negi M & Suneja P, Optimization of IAA production by endophytic *Bacillus* spp. from Vigna radiata for their potential use as plant growth promoters. *Isr J Plant Sci*, 65 (2018) 83.

41 Boby VU & Bagyaraj DJ, Biological control of root-rot of *Coleus forskohlii* Briq. using microbial inoculants. *World J Microbiol Biotechnol*, 19 (2003) 175.

42 Liu D, Chen Q, Miao Y, Wang Y & Yu K, First report of fusarium wilt of *coleus forskohlii* caused by *fusarium oxysporum* in China. *Plant Dis*, (2021).

43 Douriet-Gámez NR, Maldonado-Mendoza IE, Ibarra-Laclette E, Blom J & Calderón-Vázquez CL, Genomic analysis of *Bacillus* sp. strain b25, a biocontrol agent of maize pathogen *fusarium verticillioides*. *Curr Microbiol*, 75 (2018) 247.

44 Snook ME, Mitchell T, Hinton DM & Bacon CW, Isolation and characterization of leu7-surfactin from the endophytic bacterium *Bacillus mojavensis* RRC 101, a biocontrol agent for *Fusarium verticillioides*. *J Agric Food Chem*, 57 (2009) 4287.

45 Radhakrishnan R & Lee IJ, Gibberellins producing *Bacillus methylotrophicus* KE2 supports plant growth and enhances nutritional metabolites and food values of lettuce. *Plant Physiol Biochem*, 109 (2016) 181.

46 Hazarika SN, Saikia K, Borah A & Thakur D, Prospecting endophytic bacteria endowed with plant growth promoting potential isolated from *camellia sinensis*. *Front Microbiol*, 12 (2021) 738058.