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ANTIMICROBIAL AND CYTOTOXIC POTENTIAL OF ENDOPHYTIC ACTINOMYCETES ISOLATED FROM *Cinnamomum cassia* Presl IN LAI CHAU VIETNAM

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ABSTRACT

New antimicrobials are urgently needed to combat the threat of multidrug-resistant bacteria worldwide. Our study aimed to access the antimicrobial and cytotoxic profiles of endophytic actinomycetes associated with *Cinnamomum cassia* Presl taken from a mountainous region in the Northwest of Vietnam. The antimicrobial activity, capability for anthracycline-like compounds production and presence of secondary metabolite-biosynthetic genes were consequently determined. Finally, the cytotoxicity of antibiotic-producing actinomycetes was carried out towards various cancer cell lines. A total of 81 actinomycetes were recovered from different organs (roots, stems, leaves) of *Cinnamomum cassia*, of which 20/81 isolates exhibited antimicrobial activities against at least one of nine microbial strains. The analysis of 16S rRNA genes indicated that antibiotic-producing isolates were grouped into 4 genera *Streptomyces*, *Micromonospora, Saccharothrix,* and *Microbacterium*, among which *Streptomyces* was the most prevalent. The presence of biosynthetic genes *pks*-I, *pks*-II or *nrps* was detected in 17/20 of isolates. Five strains of *Streptomyces griseorubens* LCQ8, *Streptomyces variabilis* LCQ43, *Streptomyces californicus* LCQ44, *Streptomyces fragilis* LCQ75, and *Streptomyces beijiangensis* LCQ77 exhibited broad-spectrum antimicrobial activity against various pathogens with the MIC values ranging from 16 to 256 µg/mL. Furthermore, characterized strains showed considerable cytotoxicity against MCF-7 cell lines with a 50% inhibition concentration of crude culture extracts of less than 30 µg/mL. The findings demonstrated that *Streptomyces* species isolated from *Cinnamomum cassia* in mountainous regions hold the potential for growth inhibition of human disease agents.

Keywords: Antimicrobial activities, biosynthesis genes, cytotoxicity, *Cinnamomum cassia*, endophytes, high mountainous region, *Streptomyces*.

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INTRODUCTION

The massive use, misuse and outright abuse of antibiotics have resulted in the emergence of multidrug resistance worldwide, which is threatening global public health (Prestinaci et al., 2015). The genus *Streptomyces* has drawn global attention as the most important and promising biological source of new natural products. Due to the presence of more than 20 biosynthetic gene clusters for the production of secondary metabolites (Hopwood, 2019), this genus is able to synthesize numerous biologically important compounds, such as antibacterial, antitumoral, antiparasitic, antiviral and immunosuppressant (Rashad et al., 2015; Waksman et al., 2010; Sivalingam et al., 2019). Many studies showed that the families of actinomycetes are reported to produce about two-thirds of natural antibiotics, which *Streptomyces* accounts for 70% of available antibiotics used for the treatment of various infectious diseases (Waksman et al., 2010; Alam et al., 2022). To date, more than 1218 species with validly published names have been classified into *Streptomyces* (https://lpsn.dsmz.de/search?word=Streptomyces). Given the continual rediscovery of similar and known compounds from terrestrial actinomycetes in the last decades, exploring new areas such as medicinal plants for isolation of potential actinomycetes species is one of the successful strategies (Rashad et al., 2015; Sivalingam et al., 2019; Jiang et al., 2018).

Endophytic actinomycetes associated with medicinal plants are considered potential sources for new antibacterial and anticancer drug leads (Jiang et al., 2018; Mahdi et al., 2022). Many novel antibiotics, such as munumbicin, kakadumycin, actinomycin, and xiamycin, have been extracted from the cultured broth of endophytic *Streptomyces* strains (Castillo et al., 2003; Taechowisan et al., 2006; Ding et al., 2010). *Cinnamomum cassia* Presl is widely used as a traditional medical plant around the world especially in East and Southeast Asia. Many research have documented its significant pharmacological properties such as antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal activities (Zhang et al., 2019). Our previous study in *Cinnamomum cassia* Presl showed that 1-monolinolein and bafilomycin D from *Streptomyces cavourensis* YBQ59 are actively against multidrug-resistant bacteria and human lung cancer A-549 cells (Vu et al., 2018). Nevertheless, different ecological environments and plant genotypes greatly influence the biological diversity and distribution of endophytic species within the host plants (Gohain et al., 2015; Harrison et al., 2020). In this study, *Cinnamomum cassia* Presl plants were collected from Lai Chau province at 1,500 metres above sea level in altitude for the isolation of potential actinomycetes. The aim of this study was to screen endophytic *Streptomyces* spp. that could produce secondary metabolites for inhibition of human pathogens and cancer cell lines.

MATERIALS AND METHODS

Isolation of endophytic actinomycetes

Cinnamomum cassia samples (roots, stems and leaves) were collected from Sin Ho plateau, Lai Chau province, a Northwest high-mountain area of Vietnam (22°21’41”N, 103°16’4”E). All collected plant samples were surface sterilized, homogenized and then inoculated on nine selective media often used for the isolation of actinomycetes as previously described (Vu et al., 2020).

Identification of secondary metabolite-biosynthetic genes

The PCR screening of secondary metabolite biosynthetic genes encoding for polyketide synthase type I (PKS-I), polyketide synthase type II (PKS-II) and non-ribosomal peptide synthase (NRPS) using degenerate primers: K1F: 5’-TSA AGT CSA ACA TCGGBC A-3’ and M6R: 5’-CGC AGG TTS CSG TAC CAGTA-3’; KSaF: 5’-TSG CST GCT TGG AYG CSA TC-3’ and KSaR: 5’-TGG AAN CCG CCG AAB CCG CT-3’); A3F: 5’-GCS TACSYS ATS TAC ACS TCS GG-3’ and A7R: 5’-SAS GTCVCC SGT SCG GTA S-3’, respectively (Vu et al., 2020; Ayuso-Sacido et al., 2005). The presence of PCR amplicons with molecular weight at

approximately 1,200–1,400; 600–700 and 700–800 bp for *pks*-I, *pks*-II, and *nrps*, respectively, were recognized as positive results.

Sequencing of 16S rRNA gene and phylogeny analysis

The 16S rRNA genes of actinomycete isolates were amplified and sequenced using universal primers 27F (5'-TAACACATGCA AGTCGAACG-3') and 1429R (5'-GGTGTG ACGGGCGGTGTGTA-3') (Vu et al., 2020). The 16S rRNA gene sequence of each isolate was analyzed for the homology using a nucleotide Blast tool (http://www.ncbi.nlm.ni-h.gov/BLAST/). Neighbor-joining phylogenetic trees were constructed based on the 16S rRNA gene sequences obtained and corresponding sequences of reference strains retrieved from GenBank NCBI using MEGA7 with a branch support of 1000 bootstraps, taking *Bacillus subtilis* strain IAM 12118 as an outgroup. The 16S rRNA gene sequences of actinomycete isolates were deposited in GenBank under accession numbers MF476086 - MF476104 and KU898274.

Antimicrobial activity assay

Endophytic actinomycetes isolates were cultivated on YIM38 broth medium under shaking conditions at 200 rpm, at 30 ^{o}C for 5 days. Then, *Streptomyces*-produced compounds of the culture broth were extracted with ethyl acetate. The minimum inhibitory concentration (MIC) of the ethyl acetate extract of culture supernatants (EAEC) was determined using microdilution assay as described previously (Balouiri et al., 2016; Vu et al., 2020) against nine microbial strains including *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11105, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* Typhimurium ATCC 14028, *Bacillus cereus* ATCC 11778, *Proteus vulgaris* ATCC 49132, *Sarcina lutea* ATCC 9341, methicillin-resistant *Staphylococcus epidermidis* ATCC 35984 and *Candida albicans* ATCC 10231. These microbial strains are available at the Vietnamese Center for Conservation Microbes (VCCM), the Institute of Biotechnology, Vietnam Academy of Science and Technology. Briefly, fresh bacterial suspensions were prepared using Mueller Hinton broth (MHB) and adjusted to reach a cell density of about 5×10^8 CFU/mL. Then, 180 µL of bacterial suspensions were distributed in each well of 96-well microplates, followed by adding 20 µL of the EAEC tested. Two-fold dilution of EAEC was prepared to obtain final concentrations including 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/mL. Ethyl acetate was used as a negative control. The plates were incubated at 37 ^{o}C for 18–24 hours, afterward the cell density was determined at OD_{600} using a spectrophotometer. All the tests were performed in triplicates. The MIC is the lowest concentration of EAEC that inhibits at least 90% growth of the microbial strains.

Cytotoxicity assay

The cytotoxic effects of EAEC of bioactive actinomycetes were carried out towards Hep3B (human hepatoma), MCF-7 (human breast adenocarcinoma) and A-549 (human lung adenocarcinoma epithelial) cell lines by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Vu et al., 2020). These cell lines were kindly provided by Prof. Jeong-Hyung Lee, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Korea. Cells are cultured and maintained at 37 ^{o}C in 5% carbon dioxide (CO_2) in suitable media (RPMI 1640 for MCF-7 and A-549, and DMEM for Hep3B) containing 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 UI/mL), and streptomycin (100 mg/mL), then seeded at a density of 4×10^4 cells/mL (200 µL) in 96-well microplates. Different fractional extracts (final concentrations of 30 µg/mL and 100 µg/mL), positive control (Camptothecin 0.1 and 10 µM) and negative control (DMSO 0.1%) were added to the wells and incubated at 37 ^{o}C and 5% CO_2 for 48 hours. After the incubation, 20 µL of MTT (5 mg/mL) was then added in each well and continuously incubated at 37 ^{o}C for 4 hours. Afterwards, the culture medium was totally removed out of the wells and 200 µL of isopropanol was added to dissolve

formazan crystals. Finally, the optical density was measured at 570 nm using a BioTek Synergy microplate reader. The experiment was performed in triplicate. The rate of cell viability was calculated as cell viability (%) = $(1 - OD_{samle}/OD_{control}) \times 100\%$, with OD_{sample} and $OD_{control}$ being the optical densities of the samples and the control, respectively. The cell viability of less than 50% was recorded as the positive activity.

Statistical analysis

All the experiments were performed in triplicate. The data were expressed as mean ± standard deviation using Excel 2010 and XLSTAT 2016 software for analysis of one-site deviation (ANOVA). The difference between values was determined according to a Duncan test, and the *P* value ≤ 0.05 was statistically significant.

RESULTS

Isolation and characterization of antibiotics-producing actinomycetes

A total of 81 endophytic actinomycetes were recovered from roots (n = 35, 43.2%), stems (n = 28, 34.6%) and leaves (n = 18, 22.2%) of *Cinnamomum cassia* based on the visible morphological differences. The isolation of represent endophytic actinomycetes from the root of *Cinnamomum cassia* is shown in Figure 1. Out of them, twenty (24.7%) isolates displayed antimicrobial activity against at least one tested pathogen. Soluble pigments were produced by 20 bioactive isolates obtained mainly from roots and stems (80%) (Table 1). Among these, 14 isolates probably produced anthracycline-like antibiotics. Further, the presence of at least one important secondary metabolite biosynthesis gene either *pks*-I, *pks*-II or *nrps* was found in 85% of actinomycete isolates (Table 1). The *pks*-II gene was detected in 17 (85%) isolates, the *nrps* gene was found in 11 (55%) isolates, while the *pks*-I gene was detected in only three (15%) isolates. Three isolates including LCQ7, LCQ8 and LCQ97 possessed the three genes, whereas 11 other isolates had both *pks*-II and *nrps* genes.

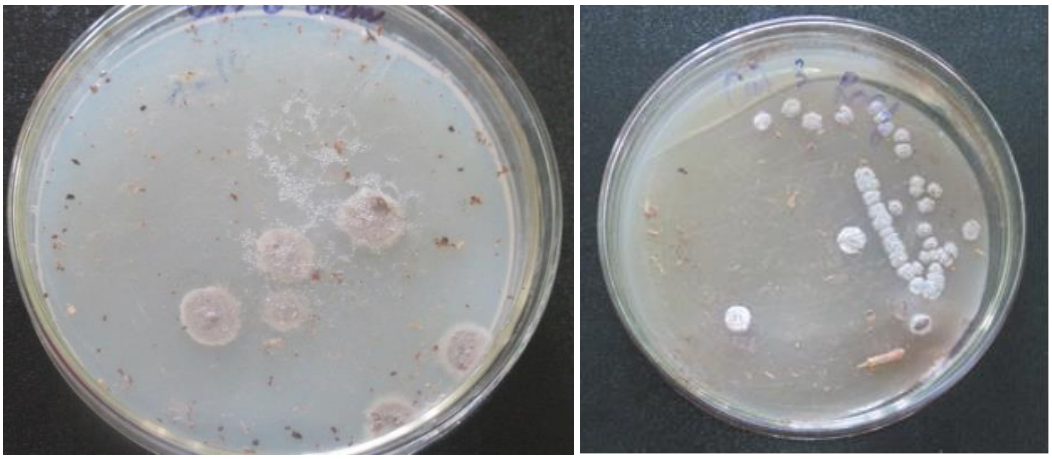

Figure 1. Isolation of endophytic actinomycetes from the root of *Cinnamomum cassia*

Identification of endophytic actinomycetes

Based on 16S rRNA gene sequence data, the majority of isolates belonged to the *Streptomyces* genus (n = 17, 85%), while rare actinomycete genera *Micromonospora*, *Saccharothrix* and *Microbacterium* were also identified (n = 1, each) (Table 1). The phylogenetic tree showed three clusters in which the main cluster consisting of all *Streptomyces* species formed two sub-clusters with various mono-branches (Fig. 2). On the other hand, the rare actinomycete genera formed separate clusters with their reference strains. Based on the phylogeny, 17 *Streptomyces* isolates were further assigned into 13 different species.

Table 1. Classification and characteristics of antibiotics-producing actinomycetes

Species	GeneBank Acc. Number	Isolation characteristics			Secondary metabolite biosynthesis gene		
		Tissue	Pigment	Anthracyclines production	*pks*-I	*pks*-II	*nrps*
Micromonospora peucetia LCQ1	MF476086	Roots	Red	-	-	+	+
Streptomyces viridodiastaticus LCQ7	MF476087	Roots	Yellow orange	-	+	+	+
Streptomyces griseorubens LCQ8	MF476088	Roots	Light yellow	+	+	+	+
Streptomyces chartreusis LCQ11	MF476089	Roots	Yellow	-	-	+	+
Streptomyces coelicolor LCQ17	MF476090	Roots	Yellow brown	-	-	+	-
Streptomyces diastaticus LCQ22	MF476091	Roots	Yellow	+	-	+	+
Streptomyces albogriseolus LCQ24	MF476092	Roots	Yellow	-	-	+	+
Microbacterium resistens LCQ33	MF476093	Roots	Yellow	-	-	+	-
Streptomyces variabilis LCQ43	MF476094	Stems	Red	+	-	+	-
Streptomyces californicus LCQ44	MF476095	Stems	Red	+	-	+	+
Streptomyces coelicoflavus LCQ47	MF476096	Stems	Yellow	+	-	+	-
Streptomyces variabilis LCQ50	MF476097	Stems	Milk white	+	-	+	+
Saccharothrix xinjiangensis LCQ61	MF476098	Leaves	Yellow gray	+	-	-	-
Streptomyces labedae LCQ69	MF476099	Leaves	White	+	-	+	+
Streptomyces fragilis LCQ74	MF476100	Leaves	Light yellow	+	-	+	-
Streptomyces coelicoflavus LCQ75	KU898274	Stems	Yellow	+	-	+	-
Streptomyces beijiangensis LCQ77	MF476101	Stems	Yellow	+	-	-	-
Streptomyces coelicolor LCQ91	MF476102	Stems	Red purple	+	+	+	+
Streptomyces sp. LCQ93	MF476103	Leaves	Yellow orange	+	-	-	-
Streptomyces variabilis LCQ97	MF476104	Stems	Gray dark	+	-	+	+
Total				14	3	17	11

Note: +, positive result; -, negative result.

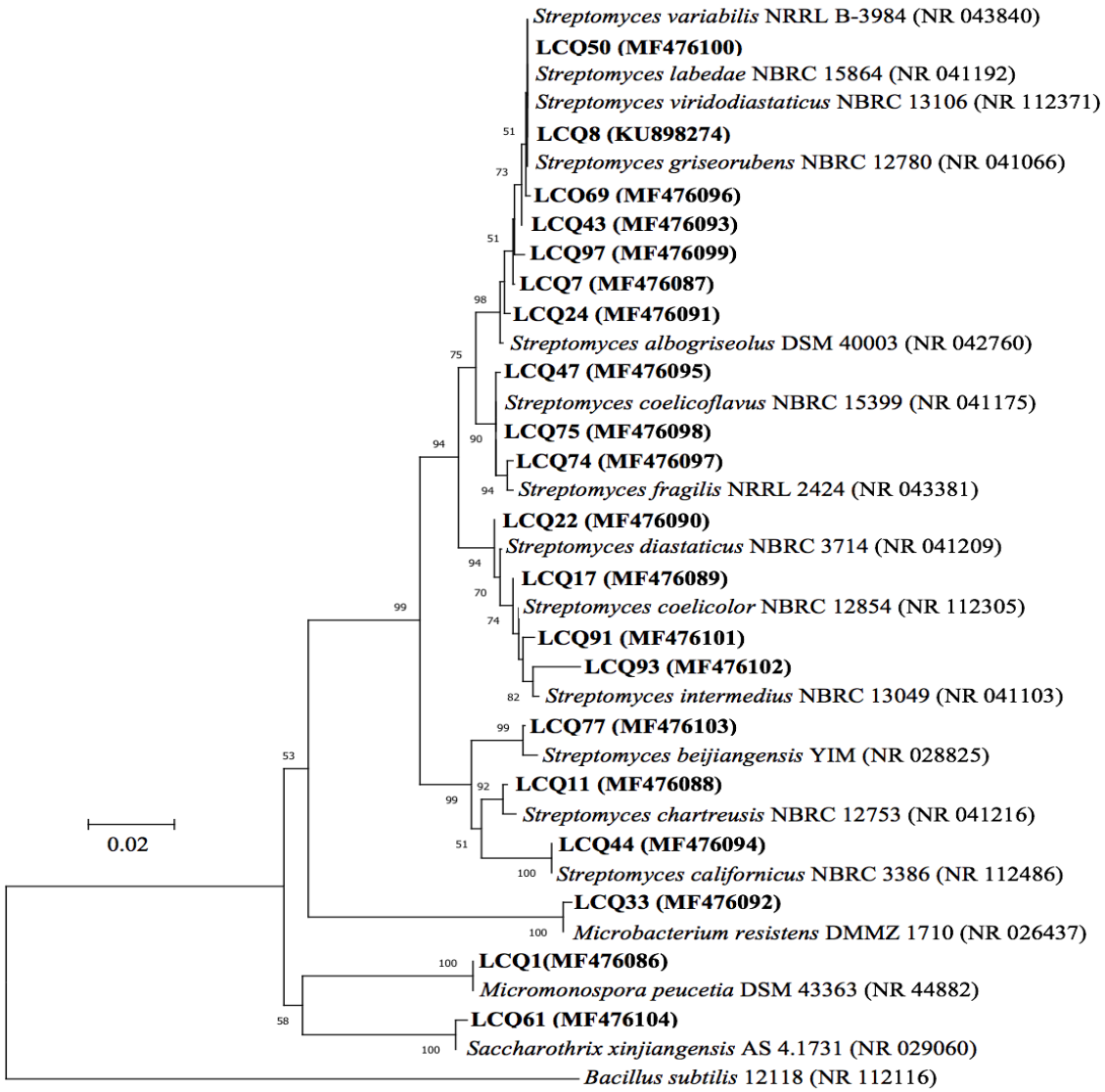

Figure 2. Neighbor-joining phylogenetic tree of 20 endophytic actinomycetes based on 16S rRNA sequence alignments with their closest reference strains (accession numbers appear in parentheses). *Bacillus subtilis* strain IAM 12118 was used as an outgroup. Scale bar indicates 0.02 substitution per nucleotide; Bootstrap values greater than 50% are shown at the nodes and are based on 1000 replicates

Antimicrobial activity towards microbial strains

Results of antimicrobial studies showed MIC values ranged from 16–256 µg/mL against the nine microbial strains tested (Table 2). They were highly active against methicillin-resistant *Staphylococcus epidermidis*, followed by *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* Typhimurium and *Pseudomonas aeruginosa*. Among broad-spectrum antimicrobial producers, *Streptomyces californicus* LCQ44 exhibited MIC values ranging from 16–256 µg/mL against eight pathogens, in which the most significant MIC values of 16 and 32 µg/mL were observed in *Candida albicans* and methicillin resistant

Staphylococcus epidermidis, respectively. *Streptomyces variabilis* LCQ43 showed MIC values ranging from 16–128 µg/mL against seven microbes and the most significant MIC value of 16 µg/mL was against methicillin-resistant *Staphylococcus epidermidis*, followed by *Pseudomonas aeruginosa*, *Salmonella enterica* Typhimurium and *Enterobacter aerogenes* (MIC value of 32 µg/mL). Isolates *Streptomyces griseorubens* LCQ8, *Streptomyces beijiangensis* LCQ77 and *Streptomyces fragilis* LCQ75 displayed MIC values of 32–256 µg/mL on five to seven microbial strains.

Table 2. Antimicrobial activity of endophytic actinomycetes against microbial strains

Isolates	Antimicrobial activity (MIC, µg/mL)								
	1	2	3	4	5	6	7	8	9
LCQ1	-	-	-	-	-	256	256	-	-
LCQ7	-	-	-	128	-	-	-	-	-
LCQ8	-	256	128	-	256	128	256	128	256
LCQ11	-	-	128	-	-	-	-	-	-
LCQ17	-	-	-	-	-	-	256	-	-
LCQ22	256	-	-	-	-	-	-	64	-
LCQ24	-	-	-	-	-	-	-	256	-
LCQ33	-	-	-	-	-	-	256	-	-
LCQ43	128	128	32	32	32	-	128	16	-
LCQ44	128	128	128	128	128	-	256	32	16
LCQ47	-	-	-	-	-	-	256	256	-
LCQ50	-	-	-	-	-	-	-	256	-
LCQ61	-	-	-	-	-	-	-	-	256
LCQ69	-	-	-	-	128	-	-	-	-
LCQ74	-	-	-	-	256	-	-	-	-
LCQ75	64	256	256	-	-	-	256	256	-
LCQ77	64	128	64	64	256	-	128	32	-
LCQ91	-	-	-	-	-	-	-	128	-
LCQ93	-	-	-	-	-	256	-	-	-
LCQ97	256	-	-	-	-	128	-	-	-
Total	6	5	6	4	6	4	9	10	3

Notes: Minimum inhibitory concentration (MIC) of the ethyl acetate extracts of actinobacterial culture broth against (**1**): *Escherichia coli* ATCC 11105; (**2**): *Proteus vulgaris* ATCC 49132; (**3**): *Salmonella enterica* Typhimurium ATCC 14028; (**4**): *Pseudomonas aeruginosa* ATCC 9027; (**5**): *Enterobacter aerogenes* ATCC 13048; (**6**): *Sarcina lutea* ATCC 9341; (**7**): *Bacillus cereus* ATCC 11778; (**8**): Methicillin-resistant *Staphylococcus epidermidis* ATCC 35984; (**9**): *Candida albicans* ATCC 10231.

Cytotoxic effects against cancer cell lines

The five broad-spectrum antibiotic producing strains *Streptomyces griseorubens* LCQ8, *Streptomyces variabilis* LCQ43, *Streptomyces californicus* LCQ44, *Streptomyces coelicoflavus* LCQ75 and *Streptomyces beijiangensis* LCQ77 were selected for the evaluation of cytotoxicity towards MCF-7, Hep3B and A-549 cancer cell lines. Overall, the EAECs show varying efficacy against cell lines in which MCF-7 was found to be the most sensitive (Table 3). When treated with 30 µg/mL, *Streptomyces fragilis* LCQ75 showed the highest cytotoxic activity toward the MCF-7 cells with cell viability of 26.9 ± 1.3%, followed by *Streptomyces griseorubens* LCQ8 (33.2 ± 1.2%),

Streptomyces californicus LCQ44 (44.3 ± 0.7%) and *Streptomyces beijiangensis* LCQ77 (47.5 ± 2.8%). Among them, only *Streptomyces griseorubens* LCQ8 and *Streptomyces beijiangensis* LCQ77 showed significant activity against Hep3B with cell inhibition found to be 19.0 ± 1.7% and 38.5 ± 1.6%, respectively at 100 µg/mL. In contrast to these results, A-547 was resistant to all treatments.

Table 3. Cytotoxic effect of the EAEC against human carcinoma cell lines

Isolates	Concentration of EAEC (µg/mL)	A-549	Hep3B	MCF-7
		% CS ± SD	% CS ± SD	% CS ± SD
Streptomyces griseorubens LCQ8	30	60.5^{c} ± 1.7	42.4^{d} ± 1.0	33.2^{b} ± 1.2
	100	53.4^{b} ± 1.5	19.0^{a} ± 1.7	27.1^{a} ± 0.7
Streptomyces variabilis LCQ43	30	76.7^{e} ± 0.9	62^{h} ± 1.9	72.1^{h} ± 1.6
	100	54.0^{b} ± 0.9	57.6^{g} ± 1.4	59.5^{f} ± 1.3
Streptomyces californicus LCQ44	30	87.0^{h} ± 0.3	99.3^{i} ± 2.0	44.3^{d} ± 0.7
	100	74.4^{e} ± 3.4	58.1^{g} ± 2.2	38.1^{c} ± 2.3
Streptomyces coelicoflavus LCQ75	30	79.6^{f} ± 1.7	56.3^{g} ± 2.2	26.9^{a} ± 1.3
	100	66.0^{d} ± 2.4	$54.8^{f,g}$ ± 2.3	24.8^{a} ± 0.6
Streptomyces beijiangensis LCQ77	30	83.1^{g} ± 0.5	51.3^{e} ± 1.2	47.5e ± 2.8
	100	61.9^{c} ± 0.7	38.5^{c} ± 1.6	38.7^{c} ± 2.5
Camptothecin (Control)	0.1 µM	76.0^{e} ± 2.2	$52.0^{e,f}$ ± 3.0	65.6^{g} ± 2.2
	10 µM	41.7^{a} ± 1.2	28.2^{b} ± 2.6	41.9^{d} ± 2.8

Notes: Cell Survival ± Standard Deviation (CS ± SD); EACE: Ethyl acetate extract of culture broth. Values with different letters are significantly different according to the Duncan test ($P < 0.05$).

DISCUSSION

The exploitation of novel antibiotics has been limited because the known compounds are often extracted from actinomycetes isolated from soil. To cope with this serious issue, switching to new sources is an effective approach to finding natural bioactive products (Rashad et al., 2015; Sivalingam et al., 2019). Undoubtedly, endophytic actinomycetes have received much attention as a promising source of novel metabolites such as antimicrobial, antiviral, anticancer, and anti-inflammatory properties (Jiang et al., 2018; Gohain et al., 2015; Vu et al., 2020). This has promoted us to continue investigating *Cinnamomum cassia* plants in order to further get a better understanding of the endophytic actinomycete community and their potential applications in medicine.

In the present study, *Streptomyces* was the most dominant genus recovered from *Cinnamomum cassia* collected in Lai Chau province, Vietnam. A total of 20 actinomycetes were classified into 4 genera, which displayed considerable activity against at least one of nine tested human pathogens. The genus *Streptomyces* showed considerable diversity, indicated by 13 different species (Fig. 1). It is consistent with global studies indicating that *Streptomyces* is the most frequently occurring genus found in other medicinal plants (Rashad et al., 2015; Gohain et al., 2015; Singh et al., 2016). In our previous study, *Streptomyces* is the predominant genus with 25 *Streptomyces* species assigned (Vu et al., 2020). The diversity of *Streptomyces* residing in *Cinnamomum cassia* collected from the high mountainous region was markedly lower than that of the low mountain area, hypothesizing that the endophytic *Streptomyces* community within *Cinnamomum cassia* plants may be geography-dependent. Hence, a culture-independent approach such as deep amplicon sequencing of the 16S rRNA gene will be an interesting subject to shed light on the actinomycete community in *Cinnamomum cassia*.

It is noteworthy that the majority of compounds secreted from *Streptomyces* spp.

are subjected to complex polyketide and non-ribosomal peptides. In this study, PCR screening of 20 endophytic actinomycetes showed that PKS-II biosynthesis distributed predominantly (85%). Similar to other habitats such as lake, coral, more than 50% of total actinomycetes are positive for the *pks*-II gene (Li et al., 2014; Zothanpuia et al., 2016; Schneemann et al., 2010). In contrast, the presence of *nrps* genes was observed at a low percentage. However, in some cases, the detected PKS and NRPS gene fragments are not relevant to the corresponding metabolites. Within our study, strain *Streptomyces beijiangensis* LCQ77 showed strong activities against 7 tested pathogenic bacteria, which did not match the negative hit for both PKS and NRPS. Schneemann et al. (2010) reported that no corresponding PKS peptides was extracted from 10 actinomycete strains, in which the presence of PKS was observed. It is more likely that secondary metabolite biosynthetic gene clusters amplified by PCR are non-functional and cryptic, leading to failure to obtain expected compounds.

As an extension to antimicrobial properties, the EAEC of five bioactive *Streptomyces* strains, including *Streptomyces griseorubens* LCQ8, *Streptomyces variabilis* LCQ43, *Streptomyces californicus* LCQ44, *Streptomyces fragilis* LCQ75 and *Streptomyces beijiangensis* LCQ77, exhibited varying levels of cytotoxic effect against cancer cell lines. MCF-7 was found to be the most sensitive to EAEC of LCQ8, LCQ44, LCQ75, and LCQ77, followed by Hep3B. In support of this, the capability of producing anthracycline-like metabolites was also observed. These evidences led to the hypothesis that these bioactive strains might produce potent anti-breast cancer agents, which induce MCF-7 cell death via p53 dependent cell death signaling pathway (Goh et al., 2014; Petitjean et al., 2007). Thus, identifying the chemical constituents present in the extracts is required for further study.

CONCLUSION

The phenotypic and genotypic data strongly indicated that endophytic *Streptomyces* associated with *Cinnamomum cassia* collected from the Northwest high-mountain area of Vietnam could be a valuable source of bioactive compounds for the treatment of human diseases. Further studies aim to predict secondary metabolite biosynthesis gene clusters through genome sequencing and identify secondary metabolite profiles.

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