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ANTIMICROBIAL AND CYTOTOXIC EFFECTS OF ENDOPHYTIC *STREPTOMYCES* STRAINS ISOLATED FROM *CINNAMOMUM CASSIA* PRESL IN VIETNAM

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SUMMARY

Vietnam is recognized as one of the countries with the high diversity of medicinal plant species in the world, nevertheless little is known about the distribution, diversity and biological activity of endophytic actinomycetes associated with host plants. The present study aimed to evaluate antimicrobial and cytotoxic activities of four endophytic *Streptomyces* strains including *Streptomyces* sp. HBQ75, HBQ87, HBQ102 and HBQ104 isolated from different organs (roots, stems or leaves) of *Cinnamomum cassia* Presl. Analysis of 16S rRNA gene sequences and the phylogenetic tree assigned them to four different *Streptomyces* species as follow *Streptomyces fulvissimus* HBQ75, *Streptomyces parvulus* HBQ87, *Streptomyces pratensis* HBQ102 and *Streptomyces ribosidificus* HBQ104. These strains exhibited broad antimicrobial spectrum against at least five out of nine pathogens tested, among them *S. parvulus* HBQ87 showed the best activity (inhibition zones >20 mm). Interestingly, *S. parvulus* HBQ87 carried all three genes (*pks*-I, *pks*-II and *nrps*) encoding for polyketide synthase or non-ribosomal peptide synthetase enzymes involved in biosynthesis of secondary metabolites, while the remaining strains only possessed one or two genes. All the *Streptomyces* strains were positive for the anthracyclines-like antibiotic activity. The cell-free supernatants of *S. parvulus* HBQ87 revealed remarkable inhibitory effects against all three human cacinomar cell lines including hepatoma Hep3B, breast adenocarcinoma MCF7 and lung cancer A549 cells at both concentrations tested (30 µg/mL and 100 µg/mL), while *S. fulvissimus* HBQ75 and *S. pratensis* HBQ102 were active against only Hep3B and MCF7 cells. In conclusion, the phenotypic and genotypic features of the four endophytic *Streptomyces* strains suggest that they have a capacity to produce different broad-spectrum secondary metabolites. Among them, *S. parvulus* HBQ87 could be the most potential candidate for the production of important antimicrobial and antitumor compounds.

Keywords: *Antimicrobial activity, antitumor activity, anthracyclines, Cinnamomum cassia, endophytic actinomycetes*

INTRODUCTION

Antibiotic plays a crucial role in the treatment of infectious diseases. However, the abuse of antibiotics has become a major factor leading to the emergence of antibiotic and multi-antibiotic resistant pathogens (Ventola, 2015). Therefore, the screening and developing new antibacterial agents having broad-spectrum antimicrobial activity, novel mode of action or multiple targets are needed to limit the emergence of multidrug resistance (Ventola, 2015). Out of 70,000 microbially-derived compounds, approximately 20,000 compounds are origined from actinomycetes (Bérdy, 2012). It is worth noting that approximately 60% of antibiotics used in clinical practices are derived from *Streptomyces* genus. This highlights that *Streptomyces* is the most important producer of valuable secondary metabolites in the nature.

Screening of endophytic actinomycetes associated with medicinal plants has gained interest

due to the interaction and evolution of them within the host plants that might lead to the production of different and novel bioactive compounds (Christina *et al.*, 2013; Matsumoto, Takahashi, 2017). Recently, many important bioactive products such as antibiotic, antifungal, antiparasitic, antiviral and antitumor agents have been isolated in endophytic actinomycetes (Christina *et al.*, 2013). Globally, endophytic *Streptomyces* genus is dominant within host plants (Golinska *et al.*, 2015). Accordingly, many novel antibiotics have been mainly found in endophytic *Streptomyces* species including munumbicins, kakadumycins, 4-arylcoumarin analogs, anthracyclines, naphthomycin K and brartemicin which were active against various serious human pathogens and different cancer cell lines (Christina *et al.*, 2013; Golinska *et al.*, 2015). Interestingly, the capacity to produce a huge number of bioactive compounds differs greatly between and within *Streptomyces* species if they are isolated from different host plants (Christina *et al.*, 2013; Golinska *et al.*, 2015; Matsumoto, Takahashi, 2017). In addition, many endophytic *Streptomyces* species possess various biosynthetic gene clusters for secondary metabolites, particularly the most important enzymes polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) (Golinska *et al.*, 2015). Thus, studying the phenotypic characteristics combined with genotypic features would gain insight into the potential of endophytic actinomycete strains associated with medicinal plants for producing new antibiotics and other bioactive compounds (Christina *et al.*, 2013; Golinska *et al.*, 2015).

Vietnam is a tropical country with variety of plant species. The country has over 3,800 medicinal plant species accounted for approximately 11% of the 35,000 species of medicinal plants known wordwide (http://vea.gov.vn/en/icorperation/Projects/Pages/the%20Biodiversity%20Partnership%20Forum%202019.aspx). This is the potential source for the isolation of endophytic actinomycetes producing valuable secondary metabolites. Unfortunately, so far very few studies on the distribution and biological characteristics of endophytic actinomycetes from medicinal plants in Vietnam have been published (Phan *et al.*, 2016; Lam, 2017; Vu *et al.*, 2019). In this context, the present study focused on the endophytic actinomycete population associated with *Cinnamomum cassia* Presl which is widely used in Vietnam as traditional medicine regimen for treating various infections and chronic diseases as well. This study aimed to evaluate antimicrobial and cytotoxic activities of different antibiotics-producing endophytic *Streptomyces* strains isolated from the host plant *C. cassia* Presl in Hoa Binh province, North Vietnam.

MATERIALS AND METHODS

According to the different morphological and biological features, four endophytic actinomycetes strains belonged to the *Streptomyces* genus including *Streptomyces* sp. HBQ75, *Streptomyces* sp. HBQ87, *Streptomyces* sp. HBQ102 and *Streptomyces* sp. HBQ104 were isolated from different organs of *C. cassia* Presl in Hoa Binh province (20°47'21''N; 105°21'20''E) were selected for the present study.

The YIM38 medium was used for studying antibiotics production of the endophytic actinomycetes strains selected. The composition of YIM38 medium (g/L) includes: malt extract 4.0; yeast extract 4.0; glucose 4.0; agar 20.0 and distilled water 1000 mL; pH 7.2 (Khieu *et al.*, 2015; Vu *et al.*, 2018).

Human pathogens including *Salmonella* Typhimurium ATCC 14028, *Escherichia coli* ATCC 11105, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 11778, *Proteus vulgaris* ATCC 49132, *Pseudomonas auroginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Enterobacter aerogenes* ATCC 13048 and methicillin-resistant *Staphylococcus epidermidis* ATCC 35984 (MRSE) were used as indicator microorganisms for the screening antimicrobial activity of cell-free supernatants from culture broth of *Streptomyces* strains using the agar well-diffusion method.

Human hepatoma Hep3B, human breast adenocarcinoma MCF7 and human lung cancer A549 cell lines were kindly prodived by Prof. Jeong-Hyung Lee, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Korea for the evaluation of cytotoxic effects of ethyl acetate extract of *Streptomyces* species. Standard antibiotic camptothecin was used as a positive control.

Screening for antimicrobial activity

Endophytic actinomycetes were cultivated in the YIM38 culture broth at 30°C with shaking 200 rpm/min, for 7 days. The YIM38 culture broth of actinomycete strains were centrifuged at 10.000 rpm/min for 10 min, then the supernatances were passed 0.22 µL filters. The cell-free supernatants of *Streptomyces* strains were used for the evaluation of

antimicrobial activity against the nine microbes using the agar well diffusion method as described before (Vu *et al.*, 2018; Vu *et al.*, 2019). The experiments were performed in triplicates.

Screening for the activity of anthracyclines-like antibiotics

The production of anthracyclines-like antibiotics of the isolated endophytic actinomycetes was screened by pigment tests as previously described (Khieu *et al.*, 2015). The mechanism of the method is as follows: Due to the presence of anthraquinone ring in the chemical composition, the color of anthracycline compounds changes depending on the pH of the environment. Follow it the orange color can be observed in acid and purple in alkaline environment. This feature is used for preliminary screening of anthracycline productive actinomycetes among the isolated actinomycete strains.

Classification of endophytic actinomycetes based 16S rRNA gene sequence and phylogenetic tree analysis

Endophytic actinomycetes were cultivated in YIM38 at 30^{o}C with shaking 200 rpm/min, for 48 hrs. The cell pellets were harvested by the centrifuge at 10.000 rpm/min in 5 min. Then, total genomic DNA was extracted as previously described by (Vu *et al.*, 2019). The amplification of 16S rRNA gene sequence of the four *Streptomyces* sp. strains was performed by using the universal primer pair 27F (5′-TAACACATGCAAGTCGAACG-3′) and 1429R (5′-GGTGTGACGGGCGGTGTGTA-3′) (Vu *et al.*, 2019). The PCR amplicons were sequenced and compared with 16S rRNA sequences in GenBank using the Blast tool (http://blast.ncbi.nlm.nihgov/Blast.cgi).

A molecular phylogenetic tree was computed using the 16S rRNA sequences of four *Streptomyces* sp. strains obtained and *Streptomyces* type strains (retrived from GenBank, NCBI). Multiple sequence alignment was performed by using CLUSTALX software and the phylogenetic tree was constructed by neighbor-joining method based on the general time reversible model using MEGA7 sofware (Kumar *et al.*, 2016). Parameters were set as a bootstrap of 1000 replications. The phylogenetic tree was rooted using *Bacillus thuringiensis* ATCC 10792 (GenBank accession number CP020754) as an out-group. The 16S rRNA gene sequences of the four *Streptomyces* strains were deposited in GenBank with the accession numbers as follow: HBQ75 (MF796970), HBQ87 (KR076807), HBQ102 (MF796968) and HBQ104 (MF796972).

Detection of biosynthetic genes

Three sets of degenerate primers: A3F (5'-GCS TAC SYS ATS TAC ACS TCS GG-3') and A7R (5'-SAS GTC VCC SGT SCG GTA S-3'), K1F (5'-TSA AGT CSA ACA TCG GBC A-3') and M6R (5'-CGC AGG TTS CSG TAC CAG TA-3'), KSaF (5'-TSG CST GCT TGG AYG CSA TC-3') and KSaR (5'-TGG AAN CCG CCG AAB CCG CT-3') were used for amplification of the *nrps*, *pks*-I and *pks*-II genes, respectively (Metsä *et al.*, 1999; Ayuso, Genilloud, 2005). PCR compositions and amplification conditions were performed as previously described (Salam *et al.*, 2017). The PCR amplicons were examined by electrophoresis on 1.5% agarose gel.

Cytotoxic assay

The cytotoxicity of ethyl acetate crude extract (EACE) of the four *Streptomyces* strains were carried out against human carcinoma cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as described previously (Khieu *et al.*, 2015; Vu *et al.*, 2018). In brief, approximately 2.5×10^4 cells/well of A549, $5x10^4$ cells/well of MCF7 and 2.5×10^4 cells/well of Hep3B were seeded into 96 well plates containing RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. After 24 hrs, different concentrations of EACE of actinomycete strains (30 and 100 µg/mL) were added into the plates and incubated at 37^{o}C, 5% CO_2 for 72 hrs. After the incubation period, 20 µL of MTT (5 mg/mL in PBS) was then added to each well and the plates were incubated at 37^{o}C, 5% CO_2 for 4 hrs. The incubation medium was discarded and 200 µL of isopropanol was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using an ExMark microplate reader (Molecular Devices, CA) and cell survival was calculated by the following formula:

Cell Viability (%) = Test OD/Control OD x 100

The tests were performed in triplicate. The cell viability value is ≤ 50% recorded as the positive activity.

Statistical analysis

The data were expressed as mean ± standard deviation using Excel 2010 and XLSTAT 2016

software for analysis of one-site deviation (ANOVA). The P value ≤ 0.05 were statistically significant.

RESULTS AND DISCUSSION

Genetic identification of *Streptomyces* species

Analysis of the 16S rRNA gene sequences of *Streptomyces* strains showed high similartities (99 – 100%) with the 16S rRNA gene sequences of corresponding reference strains retrived from GenBank through the BLAST Search tool. In agreement, the phylogenetic tree based on 16S rRNA gene sequences generated by using the neighbor-joining method indicated that, strain HBQ75 and *Streptomyces fulvissimus* strain DSM 40593T was formed a clade. Similarlity, strain HBQ87 and *Streptomyces parvulus* strain NBRC 13193T, strain HBQ102 and *Streptomyce pratensis* ATCC 33331, strain HBQ104 and *Streptomyces ribosidificus* strain NBRC 13796T were formed different clades in the different clusters of the tree (Figure 1).

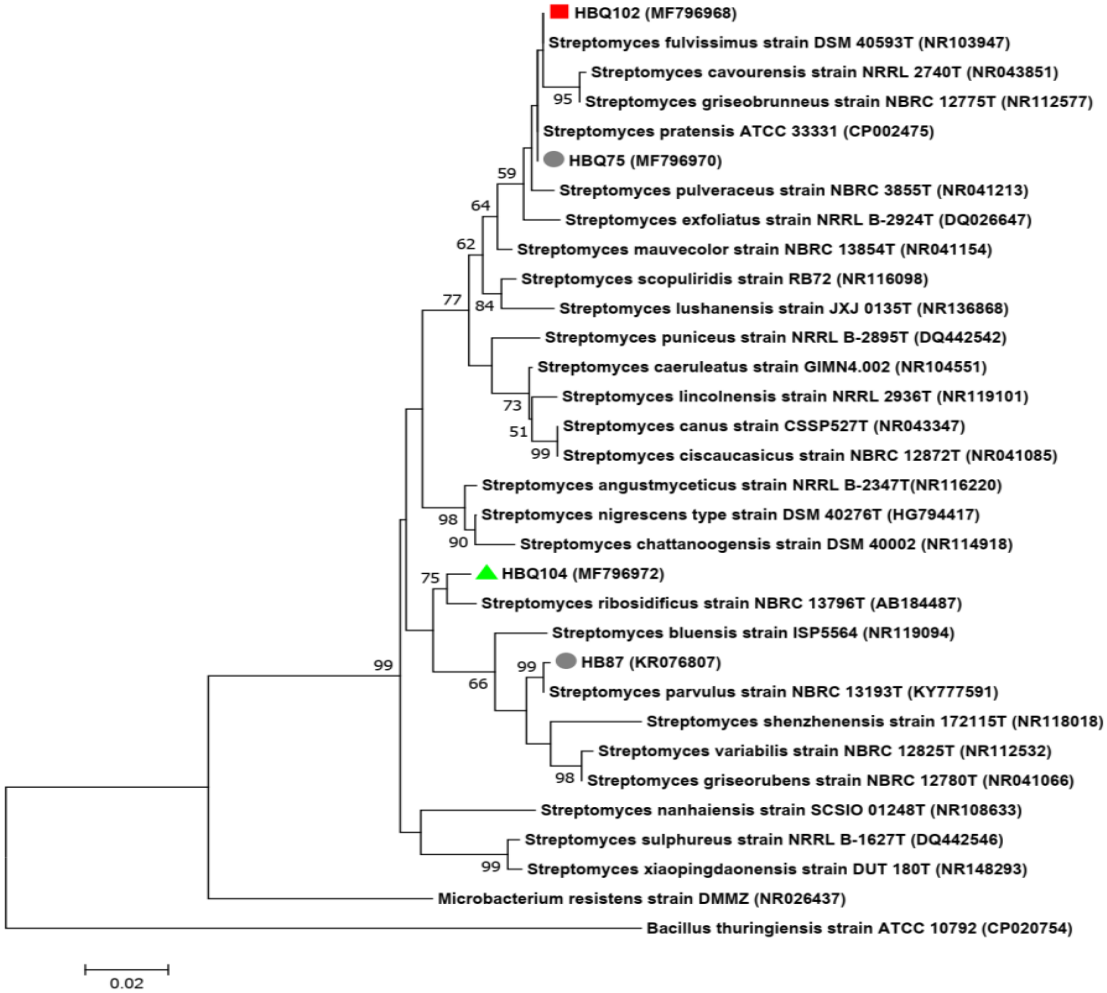

Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of *Streptomyces* strains from plants and closely related type strains. The *Bacillus thuringinesis* strain ATCC 10792 (CP020754) was used as the root of the tree. Numbers at nodes indicate the level of bootstrap support (> 50%) based on 1000 replications. The scale bar represents 20 nucleotides substitutions per 1000 nucleotides.

Acorrding to the results of 16S rRNA gene sequence; phylogenetic tree analysis, these strains were assigned to the four different species as follow *Streptomyces fulvissimus* HBQ75, *Streptomyces parvulus* HBQ87, *Streptomyces pratensis* HBQ102 and *Streptomyces ribosidificus* HBQ104. In agreement with previous studies worldwide, our study highlighted that endophytic actinomycete belonged to genus *Streptomyces* is predominant in many different medicinal plants (Qin *et al.*, 2009; Passari *et al.*, 2015). For example, Qin *et al.* (2009) found that out of 2,174 actinobacterial strains isolated from medicinal plants in a rain forest of Xishuangbanna in China, 87% were *Streptomyces* species. Similarly, a study of Passari *et al.* (2015) also showed that the *Streptomyces* genus was the most dominant, accounted for 66.6% of endophytic actinomycetes isolated from medicinal plants in India.

The antimicrobial activity of *Streptomyces* strains

Previous studies have demonstrated that endophytic actinomycetes associated with medicinal plants are potential sources of antibiotics and other valuable bioactive compounds (Golinska *et al.*, 2015; Matsumoto, Takahashi, 2017). Here, in the course of screening endophytic actinomycetes producing antibiotics, our study showed that four *Streptomyces* strains exhibited remarkable board-spectrum antimicrobial activity against 5 – 6 microbes tested. The results are shown in the Table 1 and Figure 2.

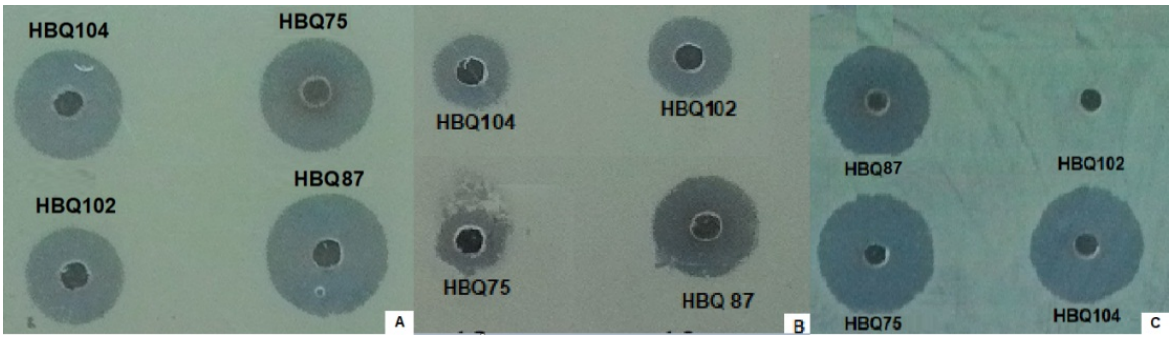

Figure 2. Antibacterial activities of cell-free supernatants of endophytic *Streptomyces* strains against *Bacillus cereus* (A), *Escherichia coli* (B) and *Sarcina lutea* (C).

Table 1. Antibacterial activities of cell-free culture supernatants of the four *Streptomyces* spieces againsts microbes.

Species (GenBank Accession No.)	Organ	Inhibition zone (D-d,mm)								
		Microbes tested								
		1	2	3	4	5	6	7	8	9
S. fulvissimus HBQ75 (MF796970)	Root	12.9^{e}±0.02	15.5^{c}±0.31	0^{f}	21.2^{a}±0.24	0^{f}	**21.0^{a}±0.11**	14.1^{d}±0.38	**18.6^{b}±0.56**	0^{f}
S. parvulus HBQ87 (KR076807)	Root	**24.2^{a}±0.09**	**20.3^{c}±0.4**	0^{e}	**21.8^{b}±0.76**	0^{e}	18.0^{d}±0.36	**25.2^{a}±0.08**	17.9^{d}±0.42	0^{e}
S. pratensis HBQ102 (MF796968)	Stem	17.4^{c}±0.05	19.6^{a}±0.05	0^{e}	19.0^{a}±0.36	0^{e}	0^{e}	18.5^{b}±0.22	15.6^{d}±0.52	0^{e}
S. ribosidificus HBQ104 (MF796972)	Leaf	13.3^{e}±0.1	16.8^{c}±0.28	0^{f}	16.1^{c}±0.27	0^{f}	14.3^{d}±0.46	20.6^{a}±0.13	17.1^{b}±0.62	0^{f}

Microbes tested*:* 1. *Escherichia coli* ATCC 11105; 2. *Proteus vulgaris* ATCC 49132; 3. *Salmonella* Typhimurium ATCC 14028; 4. *Pseudomonas aeruginosa* ATCC 9027; 5. *Enterobacter aerogenes* ATCC 13048; 6. *Sarcina lutea* ATCC 9341; 7. Methicillin-resistant *Staphylococcus epidermidis* ATCC 35984; 8. *Bacillus cereus* ATCC 11778; 9. *Candida albicans* ATCC 10231. Values with different letters are significantly different according to Fisher LCD test (P<0.05).

Specifically, all the four *Streptomyces* strains were able to inhibit the growth of three Gram-positive bacteria (*B. cereus*, *P. vulgaris* and MRSE) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), but all of them showed negative activity against the growth of *E. aerogenes*, *Salmonella* Typhimurium and *C.*

albicans (Table 1). Three strains *S. fulvissimus* HBQ75, *S. parvulus* HBQ87 and *S. ribosidificus* HBQ104 exhibited the inhibitory activity against six microbes, while *S. pratensis* HBQ102 was active against five microbes (Table 1). *S. fulvissimus* HBQ75 exhibited highest antimicrobial activities towards two microbial species including *B. cereus* and *S. lutea*, while *S. parvulus* HBQ87 showed the highest inhibitory activity against four pathogens *E. coli, P. aeruginosa*, *P. vulgaris* and MRSE. It is worth noting that *S. fulvissimus* HBQ75 and *S. parvulus* HBQ87 were isolated from the roots of *C. cassia* and they exhibited higher antimicrobial activities than *S. pratensis* HBQ102 and *S. ribosidificus* HBQ104 which were isolated from the stems and leaves of the host plant, respectively. Infact, the root of medicinal plants is the most suitable environment for the growth of endophytic microorganisms since this organ directly contacts to soils and its functions are to absorb water and nutrients from the soil (Golinska *et al.*, 2015).

In fact, many new and broad-spectrum antibiotics have been isolated from endophytic actinomycetes associated with medical plants particularly from *Streptomyces* (Qin *et al.*, 2009; Christina *et al.*, 2013; Golinska *et al.*, 2015). For instance, newly described antibiotics munumbicines isolated from endophytic *Streptomyces* NRRL 30562 associated with Australian medicinal plant *Kennedia nigriscans* were active against pathogenic fungi, *Plasmodium* species and various pathogenic bacteria including *Bacillus anthracis, Streptococcus pneumoniae, Enterococcus faecalis* (Christina *et al.*, 2013). Interestingly, these new antibiotics also had strong active against multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant strain of *Enterococcus faecalis* and multidrug-resistant *Mycobacterium tuberculosis* (Castillo *et al*., 2002). In agreement with previous studies, the results of our study underlined that endophytic actinomycete *Streptomyces* associated with medicinal plants could be potential candidates of valuable bioactive compounds.

Detection of secondary metabolite biosynthesis genes

It has been well-demonstrated that PKS and NRPS enzymes are involved in biosynthesis of valuable secondary metabolites in microorganisms such as antibitoics, antioxidase, antitumors, antimicrobials etc. (Minotti *et al.*, 2004). Therefore, in order to predict the potential for biosynthesis of important bioactive compounds, the presence of genes *pks*-I, *pks*-II encoding for PKS-I and PKS-II, respectively, and *nrps* encoding for NRPS in the four *Streptomyces* strains was investigated. Analysis of PCR results (Figure 3) showed that all the four *Streptomyces* strains possessed at least one of the biosynthetic genes (Table 2).

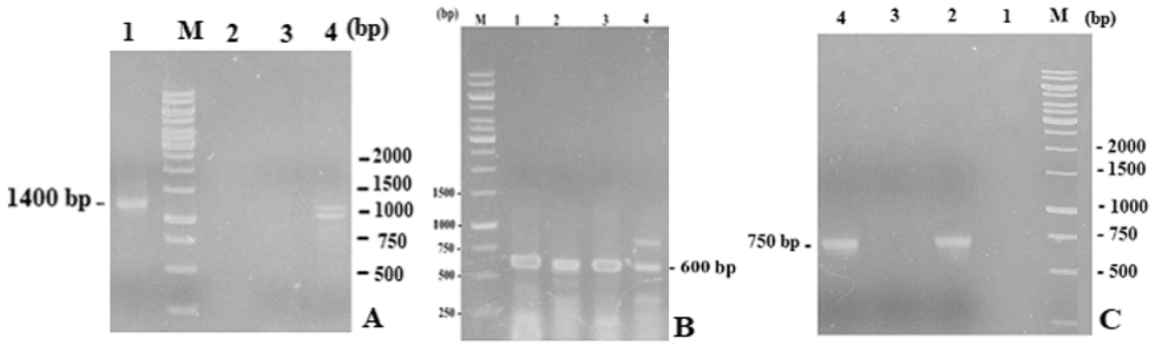

Figure 3. Agarose gel electrophoresis of the biosynthetic gene amplification products. (A) 1400 bp fragments of *pks*-I genes, (B) 600 bp fragments of *pks*-II genes, and (C) 750 bp fragments of *nrps* genes. Lane M: 1 kb molecular weight marker; lane 1: HBQ104; lane 2: HBQ75; lane 3: HBQ102; lane 4: HBQ87.

In brief, *S. parvulus* HBQ87 and *S. ribosidificus* HBQ104 were positive for the *pks*-I with approximate amplicon size of 1400 bp, all the four strains were positive for the *pks*-II (approximate amplicon size of 600 bp), while *S. fulvissimus* HBQ75 and *S. parvulus* HBQ87 possessed the *nrps* gene (approximate amplicon size of 750 bp) (Table 2). Thus, *S. parvulus* HBQ87 possessed all three biosynthetic genes, whereas *S. pratensis* HBQ102 carried only the *pks*-II gene and the remaining strains

possessed the combination of two genes *pks*-I and *pks*-II or *pks*-II and *nrps*. Since these endophytic *Streptomyces* strains have different parterns of biosynthetic genes, this genotypic data suggests that a broad array of bioactive compounds could be obtained from them. In agreement with previous studies (Qin *et al.*, 2009; Passari *et al.*, 2015), the *pks* and *nrps* genes are commonly found in the *Streptomyces*. Nevertheless, *Streptomyces* species isolated from different sources showed the different capacity of antibiotic production. The antimicrobial activities combined with the genotypic data suggest that the four endophytic *Streptomyces* species isolated from *C. cassia* would be potential candidates of novel bioactive compounds.

Table 2. The amplification results of genes encoding for PKS-I, PKS-II, NRPS enzymes from the four *Streptomyces* strains.

	Biosynthetic gene		
Species	***pks*-I**	***pks*-II**	***nrps***
S. fulvissimus HBQ75	-	+	+
S. parvulus HBQ87	+	+	+
S. pratensis HBQ102	-	+	-
S. ribosidificus HBQ104	+	+	-

Note: +: possitive result; -: negative result

Production capacity of anthracyclines-like antibiotics

Anthracyclines-like antibiotics are important antitumor agents and are widely used for cancer treatments in clinics (Nakashima *et al.*, 2013). Recently, new anthracyclines have been isolated from endophytic actinomyces, particularly in *Streptomyces* species such as *Streptomyces* sp. YIM66403, *Streptomyces scabrisporus* (Christina *et al.*, 2013; McGowan *et al.*, 2017). Using the pigment identification method, our study revealed all the four strains *S. parvulus* HBQ87, *S. pratensis* HBQ102 and *S. ribosidificus* HBQ104 positive for the production of anthracyclines-like antibiotics. In fact, anthracyclines are produced via the biosynthetic polyketide pathway and are mainly found in the *Streptomyces* (Metsä *et al.*, 2007). This result is concordant with the genotypic data since all these *Streptomyces* strains carried at least one *pks* gene. So far, the biosynthesis of doxorubicin and daunorubicin (belonged to anthracyclines) was demonstrated in *S. parvulus* species (Otten *et al.*, 1995; Han *et al.*, 2011). The finding in our study suggests that *S. parvulus* HBQ87 could able to produce the similar antitumor agents and the other *Streptomyces* species would be potential candidates of new antitumors (Trease, Evans, 1996; Zhang *et al.*, 2014; Ventola, 2015).

Cytotoxic properties of the cell-free supernatants of culture broth of endophytic *Streptomyces* strains

In order to evaluate the production capacity of antitumor agents, the cell-free supernatants (CFSs) of the four *Streptomyces* strains was used for the cytotoxic assay against three human carcinoma cell lines including A549, Hep3B and MCF7 cells (Table 3).

Among the four *Streptomyces* strains, the CFSs of three strains including *S. fulvissimus* HBQ75, *S. parvulus* HBQ87 and *S. pratensis* HBQ102 were positive for at least one type of carcinoma cell lines tested at concentration of 100 µg/mL, while *S. ribosidificus* HBQ104 was negative at the both concentrations tested. The CFSs of the three strains were positive to MCF7 cells in which the CFSs of *S. fulvissimus* HBQ75 showed highest activity (survival variability between 25% and 28% at the concentrations of 100 µg/mL and 30 µg/mL, respectively). Interestingly, at a low concentration of 30 µg/ml, *S. fulvissimus* HBQ75 and *S. parvulus* HBQ87 were active against Hep3B, while *S. fulvissimus* HBQ75, *S. parvulus* HBQ87 and *S. pratensis* HBQ102 were active against MCF7. The EACE of HBQ87 was positive for the three cell lines, also this was the only strain positive to A549 cells. *S. parvulus* HBQ87 also exhibited strong inhibitory effect against Hep3B cells (Table 3). Thus, the results obtained in this study were considerable to results in previous studies (Khieu *et al.*, 2015; Passari *et al.*, 2015). All together, the detection of three biosynthetic genes, the activity of anthracyclines-like antibiotics and cytotoxic effects towards the three different human carcinoma cell lines suggests that the *S. parvulus* HBQ87 could be the most potential candidate among the four selected endophytic *Streptomyces* species for production of valuable bioactive compounds.

Table 3. Cytotoxic effects against human carcinoma cells Hep3B, MCF7 and A549 of ethyl acetate extract of the four *Streptomoyces* species.

Species	Concentration (µg/mL)	Survival variability (SV % ± SD)		
		A549	Hep3B	MCF7
S. fulvissimus HBQ75	30	73.48^{c} ± 1.22	**47.84^{d} ± 2.18**	**28.71^{g} ± 0.74**
	100	50.84^{g} ± 1.48	**39.55^{e} ± 2.42**	**25.62^{h} ± 1.02**
S. parvulus HBQ87	30	54.22^{f} ± 2.52	**32.70^{f} ± 0.76**	**31.12^{f} ± 0.80**
	100	**37.69^{i} ± 1.04**	**31.65^{f} ± 1.25**	**25.12^{h} ± 1.28**
S. pratensis HBQ102	30	74.65$^{b.c}$ ± 0.45	52.03^{c} ± 1.64	**34.26^{e} ± 1.55**
	100	61.81^{d} ± 0.73	**47.96^{d} ± 1.98**	**25.58^{h} ± 1.28**
S. ribosidificus HBQ104	30	81.51^{a} ± 1.87	66.57^{a} ± 1.46	81.77^{a} ± 0.78
	100	56.66^{e} ± 2.85	57.58^{b} ± 1.52	77.51^{b} ± 0.25
Camptothecin	0.1 µM	76.00^{b} ± 2.27	52.03^{c} ± 3.03	65.63^{c} ± 2.20
	10 µM	**41.77^{h} ± 1.25**	**28.27^{g} ± 2.64**	**41.92^{d} ± 2.85**

SV ± SD: Survival variability ± Standard deviation. Values ≤ 50% was considered as positive activity (highlighted in the table). Values with different letters are significantly different according to Fisher LCD test ($P < 0.05$).

CONCLUSION

The endophytic actinomycete strains *S. fulvissimus* HBQ75, *S. parvulus* HBQ87, *S. pratensis* HBQ102 and *S. ribosidificus* HBQ104 exhibited broad-spectrum antibacterial activities against at least five different pathogens, had considerable cytotoxic activities towards different human carcinoma cell lines and possessed genes involved in biosynthetic pathways of secondary bioactive metabolites. Among them, *S. parvulus* HBQ87 was proved to be the potential producer of valuable secondary metabolites. Further study needs to isolate and elucidate structures of bioactive compounds derived from *S. parvulus* HBQ87.

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HOẠT TÍNH KHÁNG KHUẨN VÀ GÂY ĐỘC TẾ BÀO CỦA XẠ KHUẨN NỘI SINH *STREPTOMYCES* PHÂN LẬP TỪ CÂY QUẾ (*CINNAMOMUM CASSIA* PRESL) Ở VIỆT NAM

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TÓM TẮT

Việt Nam được xếp vào nhóm quốc gia có độ đa dạng cây dược liệu cao trên thế giới, tuy nhiên rất ít nghiên cứu về sự phân bố, mức độ đa dạng và hoạt tính sinh học của xạ khuẩn nội sinh trên cây dược liệu. Mục tiêu của

nghiên cứu này là nhằm đánh giá hoạt tính kháng khuẩn và gây độc tế bào của các chủng xạ khuẩn nội sinh thuộc nhóm *Streptomyces* gồm *Streptomyces* sp. HBQ75, HBQ87, HBQ102 và HBQ104 được phân lập từ những bộ phận khác nhau (rễ, thân hoặc lá) của cây quế *Cinnamomum cassia* Presl. Phân tích trình tự gene 16S rRNA và cây phả hệ cho thấy chúng thuộc bốn loài xạ khuẩn khác nhau là *Streptomyces fulvissimus* HBQ75, *Streptomyces parvulus* HBQ87, *Streptomyces pratensis* HBQ102 và *Streptomyces ribosidificus* HBQ104. Các chủng này có hoạt tính kháng khuẩn phổ rộng ức chế sự phát triển của ít nhất 5/9 chủng vi sinh vật được thử nghiệm, trong đó *S. parvulus* HBQ87 thể hiện hoạt tính tốt nhất (đường kính vòng ức chế > 20 mm). *S. parvulus* HBQ87 mang cả ba gen *pks*-I, *pks*-II và *nrps*, trong khi ba chủng còn lại chỉ mang từ một đến hai gen. Bốn chủng *Streptomyces* đều có khả năng sinh kháng sinh thuộc nhóm anthracyclines. Dịch chiết lên men *S. parvulus* HBQ87 ức chế sinh trưởng các dòng tế bào ung thư gồm Hep3B, MCF7 và A549 ở cả hai nồng độ thử nghiệm (30 µg/mL và 100 µg/mL), trong khi *S. fulvissimus* HBQ75 và *S. pratensis* HBQ102 ức chế các tế bào Hep3B và MCF7. Như vậy, các đặc điểm kiểu hình và kiểu gen của bốn chủng *Streptomyces* cho thấy, chúng có khả năng sinh tổng hợp các hoạt chất sinh học phổ rộng khác nhau. *S. parvulus* HBQ87 là chủng có tiềm năng nhất để sinh hợp chất kháng khuẩn và kháng tế bào ung thư.

Từ khóa: *Anthracyclines, cây quế, hoạt tính kháng khuẩn, kháng tế bào ung thư, xạ khuẩn nội sinh*