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Diversity and antimicrobial activity of culturable endophytic actinobacteria associated with Acanthaceae plants

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ABSTRACT: In this study, a total of 52 endophytic actinobacteria were isolated from 6 species of Acanthaceae plants collected in Thailand. Most actinobacteria were obtained from the root part. Based on 16S rRNA gene analysis and phylogenetic tree, these actinobacteria were classified into 4 families (*Nocardiaceae*, *Micromonosporaceae*, *Streptosporangiaceae* and *Streptomycetaceae*) and 6 genera including *Actinomycetospora* (1 isolate), *Dactylosporangium* (1 isolate), *Nocardia* (3 isolates), *Microbispora* (5 isolates), *Micromonospora* (10 isolates) and *Streptomyces* (32 isolates). The result of antimicrobial activity screening indicated that 8 isolates, including 1 *Actinomycetospora* and 7 *Streptomyces*, exhibited antimicrobial activity against tested microorganisms. In addition, the selected *Streptomyces* sp. 5R010 showed antagonistic activity against fungal plant pathogens including *Fusarium* sp., *Colletotrichum* sp. and *Sclerotium* sp. Therefore, this study demonstrated that the Acanthaceae plant species harbored the endophytic actinobacteria which can be used as the source of the antimicrobial compound.

KEYWORDS: endophytic actinobacteria, antimicrobial activity, Acanthaceae, phytopathogenic fungi

INTRODUCTION

Microorganisms, especially actinobacteria, are the primary source of the bioactive natural products which is driving drug discovery [1]. In the past century, numerous actinobacteria have been isolated from soil and used as the producer of key drugs such as actinomycin, avermectin, erythromycin, gentamicin, neomycin, platensimycin, streptomycin and vancomycin. Although many drugs are developed from the actinobacteria, the discovery of novel lead compounds has decreased because of the redundancy of the samples. Consequently, it is extremely necessary to investigate the untapped microorganisms to drive natural product research.

Actinobacteria are well known to contain valuable economically important microorganisms for a long time because of their ability to produce a large number of bioactive secondary metabolites [2]. Actinobacteria are one of the major soil microbiota. However, they are widely distributed in other various environments such as marine sediment, freshwater, insects and plants. In the past decade, the untapped habitats, especially endophytic, have become a promising source of novel actinobacteria [3].

Endophytes are the microorganisms that spend at least parts of their life cycle inside the plant tissues without having a negative impact on the host plants [4]. These microbes, especially actinobacteria, have a massive potential to produce a number of novel compounds that find wide-range application as agrochemicals, antibiotics, immunosuppressants, antiparasitics and anticancer agents [5]. A huge diversity of secondary metabolites of actinobacteria may occur because of the natural adaptation to the environments [6]. Recently, many of novel actinobacteria such as *Asanoa endophytica*, *Phytoactinopolyspora endophytica*, *Phytohabitans kaempferiae* and *Streptomyces oryzae* have been isolated from various plant species [7–10].

Acanthaceae is a family of dicotyledonous flowering plants containing approximately 210 genera and nearly 4000 species. These plants are widely distributed in tropical and subtropical regions [11]. At present, many plant species in this family, for example *Andrographis paniculata*, *Barlelia lupulina*, *Clinacanthus nurans* and *Thunbergia laurifolia*, have been used for Thai traditional medicines. However, the actinobacteria associated with this plant family are rarely reported. Therefore, the objectives of this study were to study the diversity of endophytic actinobacteria associated with the Acanthaceae plant and to screen the antimicrobial activity of the actinobacterial isolates

MATERIALS AND METHODS

Plant collections and isolation of actinobacteria

Plant samples were collected and planted in the botanical garden of the Department of Biology, Faculty of Science, Ramkhamhaeng University prior to isolation. In this study, 6 species of plants in the Family Acanthaceae including *Andrographis paniculata*, *Asystasia gangetica*, *Berleria lupulina*, *Clinacanthus nutans*, *Justicia subcoriacea* and *Ruellia squarrosa* were collected.

Actinobacteria were isolated from leaves, stems, and roots of each plant sample. Plant samples were washed to remove soil from the samples. The three-step surface sterilization was used to eliminate the surface microbes. Briefly, a 5-min wash in 3% NaOCl, followed by a 1-min wash in 95% ethanol and a final wash with a sterile distilled water 2 times. A 0.5 g of the surface-sterilized materials was aseptically ground with 5 ml of extraction solution [12]. Then, 0.1 ml of plant suspension was spread on humic acid-vitamin agar [13], starch casein nitrate agar [14] and proline agar [15] supplemented with nalidixic acid (25 mg/l) and cycloheximide (50 mg/l) to control the growth of Gram-negative bacteria and fungi, respectively. The plates were incubated at 30 °C for 14 days. The colonies of actinobacteria were collected and purified on ISP2 medium.

Identification of actinobacteria

The identification of actinobacteria was performed by 16S rRNA gene analysis. The genomic DNA of actinobacteria was extracted from the mycelia grown in yeast-dextrose broth (1 g glucose; 1 g yeast extract; 100 ml water, pH 7.0–7.2) at 30 °C for 3–7 days [16]. The amplification was carried out using standard primers (5′-GAGTTTGATCCTGGCTCAG-3′) and 1530R (5′-GTTACCTTGTTACGACTT-3′) with the initial incubation of 3 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C and followed by a 3 min final extension at 72 °C [17, 18]. The nucleotide of the PCR product was sequenced using the sequencing service (Macrogen, Korea). The nucleotide sequence was manually analyzed using BioEdit software (Ibis Biosciences). BLAST was determined using the EzbioCloud database [19]. Phylogenetic analysis was constructed using MEGA 7.0 software [20]. The tree topology was evaluated using the bootstrap test [21].

Antimicrobial activity screening

Antimicrobial activity of actinobacterial isolates was determined using the agar disc diffusion method. Briefly, each actinobacterium was cultured in ISP2 broth pH 7.0 in shaking condition at 180 rpm 30 °C for 14 days. Then, one volume (equivalent to culture broth volume) of 95% ethanol was added and shook at 180 rpm for 1 h followed by centrifuge at 4500 rpm for 10 min. The supernatant was collected and preserved at −20 °C. To prepare the tested disc, the sterile paper disc was dipped into each broth library and air-dried in the biosafety cabinet. The sterile ISP2 broth added with one volume of ethanol was used as the negative control.

Six microorganisms including 3 Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and *Kocuria rhizophila*, and 2 Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*, and a yeast, *Candida albicans*, were used as the tested microorganisms. The tested bacteria and yeast were activated on Mueller-Hinton agar (MHA) and sabouraud dextrose agar (SDA) for 27 h at 37 °C and 30 °C, respectively. To prepare a microbial suspension, the turbidity of each tested microorganism in normal saline solution was adjusted to 0.5 McFarland standards. Then, the tested bacteria and yeast were swabbed on the surface of MHA and SDA, respectively. The prepared paper disc was put on the surface of media swabbed with the tested microorganisms and incubated for 24 h at 37 °C and 30 °C for bacteria and yeast, respectively. The inhibition zone was observed and documented.

Antagonistic activity against phytopathogenic fungi of the selected strain

The co-cultivation method was used to determine the antagonistic activity of the selected actinobacteria against 6 phytopathogenic fungi including *Colletotrichum gloeosporioides*, *Colletotrichum* sp.,

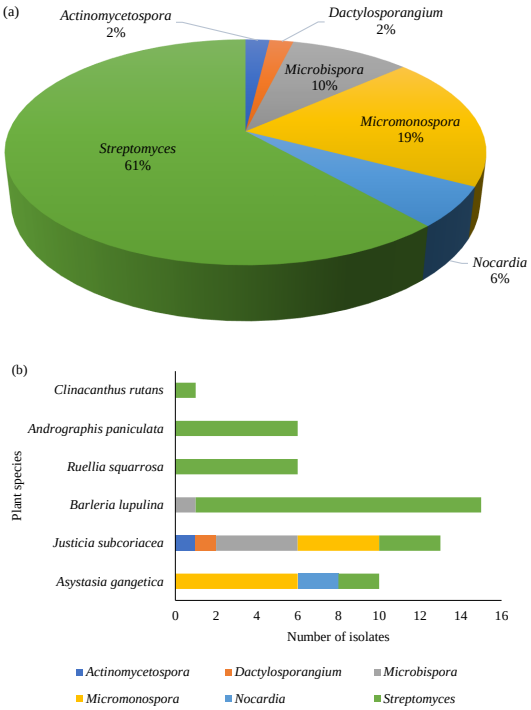

Fig. 1 Diversity of actinobacteria isolated from Acanthaceae plant species. (a) Pie chart represented the percentage of actinobacterial genera within the total number of isolates. (b) The number of actinobacteria isolated from different plant species.

Curvularia oryzae, *Fusarium* sp., *Lasiodiplodia theobromae* and *Sclerotium* sp.

The selected actinobacterium was cultured on one side of the ISP2 agar plate and incubated at 30 °C for 7 days. Then, the 7-day-old of the tested phytopathogenic fungi grown on SDA agar were cut by the cork borer (6 mm in diameter) and transferred to the opposite of the prepared actinobacterium plate and incubated at 30 °C for 7–10 days. The inhibition zone around the actinobacterial colony indicated fungal inhibition. The fungi grown on ISP2 agar without actinobacteria were used as the growth control of the fungi.

RESULTS AND DISCUSSION

Diversity of actinobacteria

In this study, 52 actinobacteria were isolated from leaves, stems and roots of 6 species of Acanthaceae plants. In this number, 49 isolates were obtained from roots, followed by 2 and 1 isolate obtained from leaves and stem, respectively. The results of this study are similar to the previous studies showing that nearly all the plants harbor endophytes [22]. Janso and Carter [23] discussed that actinobacteria could be isolated from every tissue type of samples; however, root and bark had the highest isolate-to-sample ratio.

On the basis of BLAST result and phylogenetic tree analysis, actinobacteria obtained in this study were identified and categorized into 4 families (*Nocardiaceae*, *Micromonosporaceae*, *Streptosporangiaceae* and *Streptomycetaceae*) and 6 genera including *Actinomycetospora* (1 isolate), *Dactylosporangium* (1 isolate), *Nocardia* (3 isolates), *Microbispora* (5 isolates), *Micromonospora* (10 isolates) and *Streptomyces* (32 isolates) (Figs. 1 and 2, Table 1). Based on this study, the most abundant genus found in Acanthaceae plants were *Streptomyces* (61%) followed by *Micromonospora* (19%) and *Microbispora* (10%) (Fig. 1). The pattern of the diversity of culturable actinobacteria of this study, of which *Streptomyces* are the predominant species, is similar to the previous report [24]. In 2012, Kim et al [25] isolated 61 endophytic actinobacteria, comprising 15 genera including *Streptomyces*, *Micromonospora*, *Rhodococcus*, *Microbispora*, *Micrococcus*, *Microbacterium*, *Streptacidiphilus*, *Arthrobacter*, *Dietzia*, *Kitasatospora*, *Herbiconiux*, *Mycobacterium*, *Nocardia*, *Rathayibacter* and *Tsukamurella*, from the native herbaceous plant species of Korea. In that study, they found that members of the genus *Streptomyces* comprised 45.9% of the total isolates and were followed by *Micromonospora* (18.8%). In the study of Janso and Carter [23], 123 isolates of endophytic actinobacteria, including 17 genera, were isolated from the tropical native plants in Papus New Guinea and Mborokua Island, Solomon Island. The community of endophytic actinobacteria may vary according to the host plant. Jiang et al [26] isolated 101 endophytic actinobacteria from 5 different mangrove plants including *Avicennia marina*, *Aegiceras corniculatum*, *Kandelia obovota*, *Bruguiera gymnorrhiza* and *Thespesia populnea*. Based on 16S rRNA gene, these actinobacteria were distributed in 15 families and 28 genera including *Actinoplanes*, *Agrococcus*, *Amnibacterium*, *Brachybacterium*, *Brevibacterium*, *Citricoccus*, *Curtobacterium*, *Dermacoccus*, *Glutamicibacter*, *Gordonia*, *Isoptericola*, *Janibacter*, *Kineococcus*, *Kocuria*, *Kytococcus*, *Leucobacter*, *Marmoricola*, *Micrococcus*, *Microbacterium*, *Micromonospora*, *Mycobacterium*, *Nocardioides*, *Nocardia*, *Nocardiopsis*, *Pseudokineococcus*, *Sanguibacter*, *Streptomyces* and *Verrucosispora*. In addition, Widiantini and Franco [27] reported that the dominant

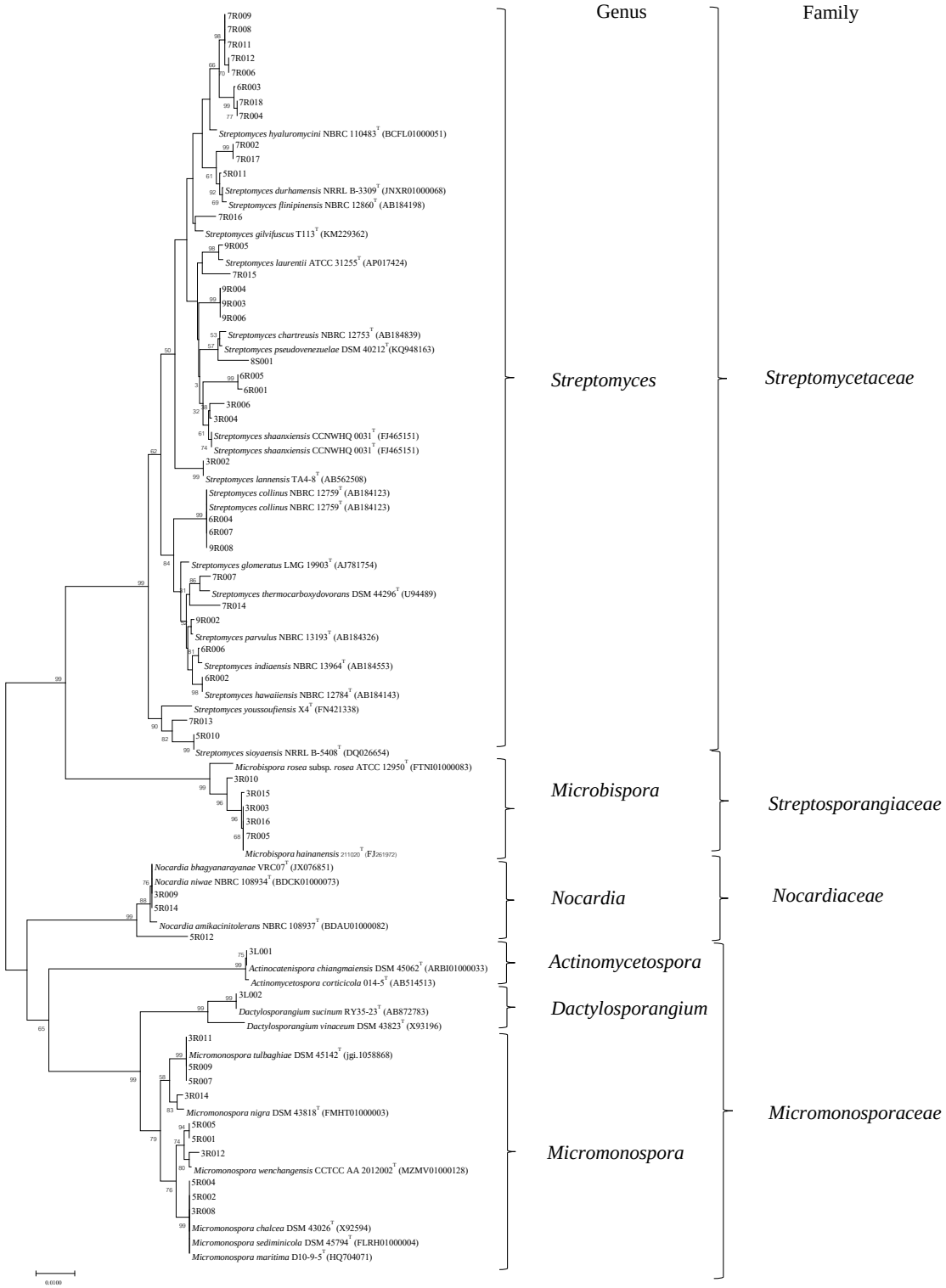

Fig. 2 Neighbor-Joining phylogenetic tree based on 16S rRNA gene of the actinobacterial isolates and closely related actinobacterial type strains shown that the isolates were clustered within 4 families and 6 genera. Numbers at the nodes indicate bootstrap values based on 1000 replicates.

Table 1 Closest BLASTN matches for the 16S rDNA sequence and antimicrobial activity of the actinobacterial isolates.

Plant host	Plant material	Isolation no.	Accession no.	BLAST match result		Isolation media	Inhibition zone (mm)					
				Closest species	Similarity %		K	B	S	P	E	C
Asystasia gangetica	Root	5R001	LC497879	*M. chokoriensis* DSM45160^T	99.51	SCN	–	–	–	–	–	–
		5R002	LC497878	*M. maritima* D10-9-5^T	100	SCN	–	–	–	–	–	–
		5R004	LC497877	*M. maritima* D10-9-5^T	99.79	SCN	–	–	–	–	–	–
		5R005	LC497876	*M. chokoriensis* DSM45160^T	99.51	SCN	–	–	–	–	–	–
		5R007	LC497873	*M. tulbaghiae* DSM 45142^T	100	SCN	–	–	–	–	–	–
		5R009	LC497875	*M. tulbaghiae* DSM 45142^T	100	Proline	–	–	–	–	–	–
		5R010	LC500018	*S. sioyaensis* NRRL-B5408^T	99.72	HV	–	–	–	–	–	16
		5R011	LC497872	*S. durhamensis* NRRL-B3309^T	99.41	HV	26	15	17	–	–	–
		5R012	LC497874	*N. xishanensis* NBRC 101358^T	99.93	HV	–	–	–	–	–	–
		5R014	LC497871	*N. bhagyanarayanae* VRC07^T	98.68	SCN	–	–	–	–	–	–
Justicia subcoriacea	Root	3R002	LC497888	*S. lannensis* TA4-8^T	99.93	SCN	–	–	–	–	–	–
		3R003	LC497882	*M. hainanensis* 211020^T	100	HV	–	–	–	–	–	–
		3R004	LC497890	*S. shaanxiensis* CCNWHQ0031^T	99.30	HV	21	21	27			
		3R006	LC497880	*S. cyaneus* NRRL B-2296^T	98.91	HV	–	–	–	–	–	–
		3R008	LC497886	*M. chalcea* DSM 43026^T	99.65	proline	–	–	–	–	–	–
		3R009	LC497889	*N. bhagyanarayanae* VRC07^T	99.72	proline	–	–	–	–	–	–
		3R010	LC497887	*M. hainanensis* 211020^T	99.17	proline	–	–	–	–	–	–
		3R011	LC497885	*M. tulbaghiae* DSM 45142^T	99.44	SCN	–	–	–	–	–	–
		3R012	LC497891	*M. wenchangensis* CCTCCAA 2012002^T	99.44	SCN	–	–	–	–	–	–
		3R014	LC497881	*M. nigra* DSM 43818^T	98.25	SCN	–	–	–	–	–	–
		3R015	LC497884	*M. hainanensis* 211020^T	99.72	HV	–	–	–	–	–	–
		3R016	LC497883	*M. hainanensis* 211020^T	99.72	HV	–	–	–	–	–	–
	Leaf	3L001	LC497892	*Actinomycetospora corticicola* 014-5^T	99.62	Proline	22	19	18	–	–	–
		3L002	LC497920	*Dactylosporangium sucinum* RY35-23^T	99.58	Proline	–	–	–	–	–	–
Barleria lupulina	Root	7R002	LC497919	*S. shenzhenensis* 172115^T	99.38	HV	–	–	–	–	–	–
		7R004	LC497918	*S. shenzhenensis* 172115^T	99.65	Proline	–	–	–	–	–	–
		7R005	LC497917	*M. hainanensis* 211020^T	99.86	Proline	–	–	–	–	–	–
		7R006	LC497916	*S. graminisoli* JR-19^T	99.93	HV	–	–	–	–	–	–
		7R007	LC497908	*S. chiangmaiensis* T4A-1^T	98.75	HV	–	–	–	–	–	–
		7R008	LC497915	*S. graminisoli* JR-19^T	99.51	HV	–	–	–	–	–	–
		7R009	LC497914	*S. graminisoli* JR-19^T	99.86	HV	–	–	–	–	–	–
		7R011	LC497913	*S. graminisoli* JR-19^T	99.86	starch	–	–	–	–	–	–
		7R012	LC497912	*S. graminisoli* JR-19^T	99.79	proline	–	–	–	–	–	–
		7R013	LC497911	*S. lilacinus* NRRL 1968^T	99.21	proline	15	–	–	–	–	–
		7R014	LC497910	*S. lusitanus* NBRC 13464^T	99.65	HV	–	–	–	–	–	–
		7R015	LC497906	*S. neopeptinius* KNF 2047^T	98.64	HV	–	–	–	–	–	–
		7R016	LC497907	*S. gilvifuscus* KM229362^T	98.21	HV	–	–	–	–	–	–
		7R017	LC500017	*S. shenzhenensis* 172115^T	99.45	HV	–	–	–	–	–	–
		7R018	LC497909	*S. shenzhenensis* 172115^T	99.58	HV	–	–	–	–	–	–
Ruellia squarrosa	Root	9R002	LC497898	*S. parvulus* NBRC 13193^T	99.72	HV	–	–	–	–	–	–
		9R003	LC497897	*S. chartreusis* NBRC 12753^T	99.31	SCN	9.5	8	7	–	–	–
		9R004	LC497895	*S. chartreusis* NBRC 12753^T	99.38	proline	8.5	8	–	–	–	–
		9R005	LC497896	*S. laurentii* ATCC 31255^T	99.31	HV	–	–	–	–	–	–
		9R006	LC497894	*S. chartreusis* NBRC 12753^T	99.38	proline	–	–	–	–	–	–
		9R008	LC497893	*S. collinus* NBRC 12759^T	99.93	HV	19	12	14	–	–	–
Andrographis paniculata	Root	6R001	LC497905	*S. deccanensis* DAS-139^T	99.78	HV	–	–	–	–	–	–
		6R002	LC497904	*S. hawaiiensis* NBRC 12784^T	99.72	HV	–	–	–	–	–	–
		6R003	LC497903	*S. shenzhenensis* 172115^T	99.79	HV	–	–	–	–	–	–
		6R004	LC497902	*S. collinus* NBRC 12759^T	99.93	HV	–	–	–	–	–	–
		6R005	LC497901	*S. deccanensis* DAS-139^T	99.79	HV	–	–	–	–	–	–
		6R006	LC497900	*S. indiaensis* NBRC 13964^T	99.29	HV	–	–	–	–	–	–
Clinacanthus rutans	Stem	8S001	LC500016	*S. cavourensis* NBRC 13026^T	100	HV	–	–	–	–	–	–

K = *Kocuria rhizophila*; B = *Bacillus subtilis*; S = *Staphylococcus aureus*; P = *Pseudomonas aeruginosa*; E = *Escherichia coli*; C = *Candida albicans*.

endophytic actinobacteria species isolated from rice plants of Australia is *Microbispora*. The variable of endophytic actinobacterial species in the different plants may depend on factors such as host specificity, stage of the host, type of sample, geographical condition, season, surface sterilant, culture condition and selective media [28, 29].

Antimicrobial activity

In this study, 8 isolates including 1 *Actinomycetospora* and 7 *Streptomyces* exhibited antimicrobial activity against tested microorganisms. Most of the active isolates showed antimicrobial activity against Gram-positive bacteria, but no activity was observed

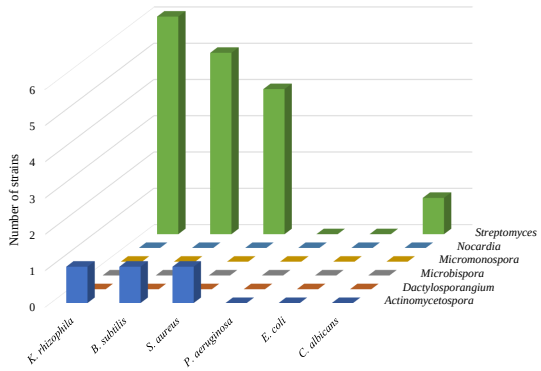

Fig. 3 Antimicrobial activity of the actinobacteria against tested microorganisms.

against Gram-negative bacteria (Fig. 3, Table 1). The antimicrobial activity of endophytic *Streptomyces* against Gram-positive bacteria has been documented in previous studies. Zhang et al [30] studied antimicrobial activity of 65 endophytic actinobacteria, isolated from *Achyranthes bidentata*, *Paeonia lactiflora*, *Radix Platycodi* and *Artemisiae argyi*, against *penicillin resistant Staphylococcus aureus*. They found that 12 strains, the majority of which were *Streptomyces* spp., showed activity against this pathogen. Although no actinobacteria obtained from this study showed anti-Gram-negative bacterial activity. Mingma et al [31] isolated 317 actinobacteria from root and rhizospheric soils of leguminous plants, and 64 of the isolates (20.2%) showed antagonistic activity against soybean pathogen *Xanthomonas campestris* pv. glycine. In addition, 21 endophytic actinobacteria isolated by Jiang et al [26] showed activity against *P. aeruginosa*. This evidence showed that anti-Gram-negative bacteria could be observed in some endophytic actinobacteria.

The production of novel antimicrobial metabolites from endophytic actinobacteria has been documented in the various reports. These include maklamicin, misamycin and diastaphenazine.

Maklamicin, a new spirotetronate-class polyketide isolated from *Micromonospora* sp. GMKU326 — the endophytic actinobacteria from root nodule of the legume *Lupinus angustifolius*, showed strong to moderate antimicrobial activity against Gram-positive bacteria including *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus* and *Enterococcus faecalis* with MIC values of 0.2, 1.7, 6.5, 13 and 13 μg/ml, respectively [32].

Misamycin, a new anthracycline antibiotic, was isolated from the culture broth of endophytic *Streptomyces* sp. YIM66403. The compound exhibited moderate antibacterial activity against *S. aureus* with MIC value of 64 μg/ml. Besides antibacterial activity, it showed cytotoxicity against various human cell lines including human promyelocytic leukemia HL-60, human hepatoma SMMC-7721, non-small cell long cancer A-549, breast cancer MCF-7 and human colorectal carcinoma SW4801 with IC_{50} values of 15.37, 16.34, 25.98, 20.71 and 9.75 μM, respectively [33].

Diastaphenazine, a new dimeric phanazine, was isolated from the culture broth of endophytic *Streptomyces diastacicus* subsp. *ardesiacus* from sterile tissue of *Artemisia annua*. The compound showed antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. In addition, it showed weak cytotoxicity against 5 human tumor cell lines including BGC-823, Hela, HCT116, HepG2 and H460 with IC_{50} values of 14.9, 28.8, 65.2, 82.5 and >100 μM, respectively [34].

In this study, the isolate 5R010, closely related to *Streptomyces sioyaensis* NRRL-B5408^T, showed antifungal activity against *C. albicans*. This isolate was selected to test the antagonistic activity against phytopathogenic fungi.

Antiphytopathogenic fungi activity

Based on the co-cultivation method, the strain 5R010 showed antagonistic activity against *Fusarium* sp., *Colletotrichum* sp. and *Sclerotium* sp., but no activity was observed on *Colletotrichum gloerosporiodes*, *Curvularia oryzae* and *Lasiodiplodia theobromae* (Fig. 4). It has been reported in several studies that the endophytic actinobacteria can be used to control plant diseases. Álvarez-Pérez et al [35] used endophytic actinobacteria isolated from the root system of the grapevine plants, *Vitis vinifera*, to reduce nursery fungal graft infections caused by *Diplodia seriata*, *Dactylonectria macrodidyma*, *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*. Taechowisan et al [36] reported that 3 endophytic *Streptomyces* sp. showed strong inhibition for *Colletotrichum musae* and 5 were very active against *Fusarium oxysporum*. The *Streptomyces* strain CEN6, isolated from *Centella asiatica*, showed good antagonistic activity against *Alternaria brassicicola*; the pathogen causes leaves spot of cabbage. The fungal treated by this stain showed abnormal characteristics including swelling and frequent septa [37]. The use of endophytic *Streptomyces platensis* F-1, isolated from *Oryza sativa*, as biofumigation to control

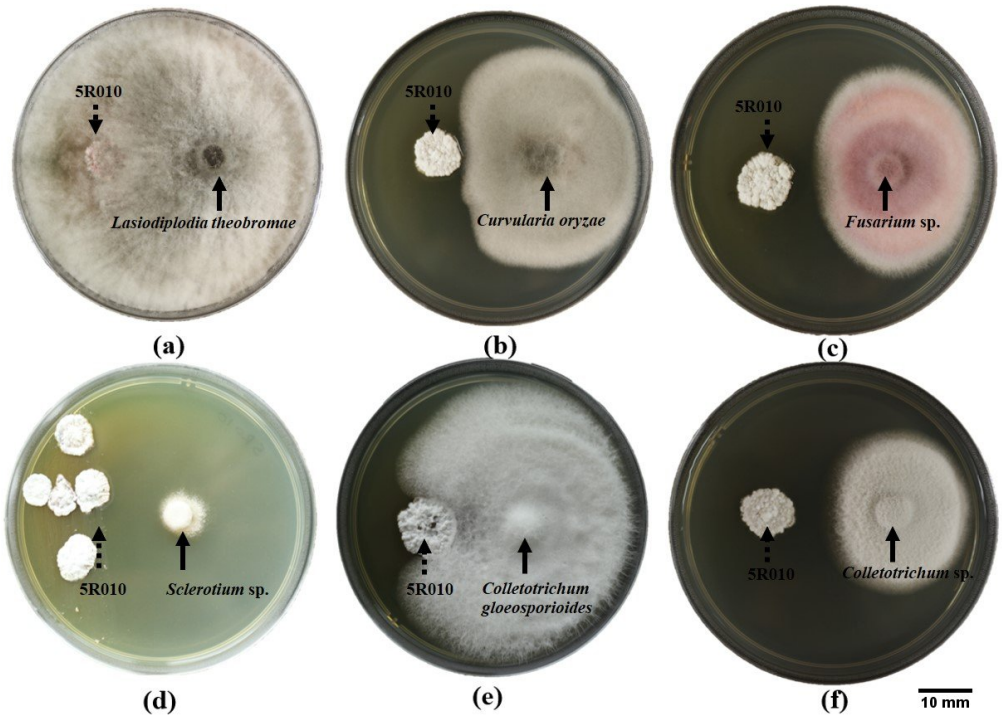

Fig. 4 Antagonistic activity of the isolate 5R010 against phytopathogenic fungi (a) *Lasiodiplodia theobromae*, (b) *Curvularia oryzae*, (c) *Fusarium* sp., (d) *Sclerotium* sp., (e) *Colletotrichum gloeosporioides* and (f) *Colletotrichum* sp. The arrows ⇢ and ⟶ indicate the colony of isolate 5R010 and fungal pathogens, respectively.

plant fungal disease was reported by Wan et al [38]. The volatile substance produced by the strain F-1 could effectively reduce the incidence and the severity of the disease caused by *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Besides the application as biocontrol, the novel antifungal compounds such as dehydroxyaquayamycin B and fistupyrone were isolated from endophytic actinobacteria. Dehydroxyaquayamycin B, a new C-glycosylated benz[α]anthraquinone, was isolated from endophytic *Streptomyces blastomycetica* F4-20. The compound showed fungicidal activity against *Valsa mali*, *Colletotrichum orbiculare* and *Fusarium graminearum* [39]. Fistupyrone, a new microbial compound, isolated from the culture broth of endophytic *Streptomyces* sp. TP-A0569 can inhibit the *in vivo* infection of the seedlings of Chinese cabbage by *Alternaria brasicicola*, the cause of *Alternaria* leaf spot [40]. The antagonistic activity of the strain 5R010 found in this study revealed that this strain may be used for the fungal biocontrol in the future. In addition, the active compounds produced by this strain should be characterized in further study.

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Authors' contributions: we declare that the present study was performed by the authors named in this article. W. Phongsopitanun designed the study and performed experiments on isolation, identification of actinobacteria and screening of antimicrobial activity of the actinobacterial isolates; P. Sripreechasak performed experiments on data analysis; K. Rueangsawang and R. Panyawut performed experiments on plant sample collection and identification of the plant species; P. Pittayakhajonwut and S. Tanasupawat gave conceptual advice.

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