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Diversity, antimicrobial activity, and susceptibility of culturable soil actinobacteria isolated from Sichang Island

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ABSTRACT: *Actinobacteria* are a promising source of novel antibiotics. The study of diverse actinobacteria from Sichang Island may lead to a discovery of new microbes with pharmaceutical applications. Some actinobacteria, especially those in the genus *Nocardia*, can cause infection in humans and animals. A total of 55 actinobacteria were isolated from six soil samples collected from Sichang Island, Chonburi Province, Thailand. Based on the morphological characteristics and 16S rRNA gene analysis, the actinobacterial isolates were classified into three genera including *Streptomyces* (32 isolates), *Nocardia* (22 isolates), and *Saccharothrix* (1 isolate). Most of the *Streptomyces* and the *Saccharothrix* isolates exhibited antimicrobial activity, with none observed among the *Nocardia* strains. The 16S rRNA gene similarity suggested three strains represented candidates of novel taxa. Moreover, most of the *Nocardia* were rare environmental species. Antimicrobial susceptibility testing revealed that amoxicillin/clavulanate (2/1), imipenem, and linezolid were active against *Nocardia* strains, but they were not susceptible to ceftriaxone, cefotaxime, clarithromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole. The susceptibility profiles vary between strains and species.

KEYWORDS: actinobacteria, antimicrobial activity, *Nocardia*, antimicrobial susceptibility, Sichang Island

INTRODUCTION

Natural products with pharmaceutical applications can be generated by primary and secondary metabolism of living things [1]. Microorganisms, especially actinobacteria, are the primary source of bioactive natural products [2]. The phylum *Actinobacteria* consisting of Gram-positive high guanine+cytosine (G+C) filamentous bacteria is one of the most diverse bacterial groups. *Actinobacteria* have diverse morphologies ranging from the unicellular rods or cocci to filamentous mycelia [3]. The microbes are widely distributed both on land and in aquatic environments. On land, the soil is the main habitat of most actinobacteria. One gram of soil can contain 10^6 to 10^9 CFU of actinobacteria [4]. Some actinobacteria are symbiotic with other creatures such as marine sponges and corals as well as plants and lichens [5, 6]. Different habitats affect the metabolic diversity of the microbes, leading to the production of various types of secondary metabolites.

Actinobacteria, especially genus *Streptomyces*, are an essential source for antibiotic discovery [7]. Their chemically bioactive secondary metabolites are diverse. To date, some 12 000 bioactive compounds have been produced by actinobacteria, and two-thirds of the known antibiotics are produced by members of the genus *Streptomyces*. *Actinobacteria* also produce many anticancer and antifungal compounds, antiviral agents, antiparasitics, insecticides, herbicides, immunosuppressants, and therapeutic enzymes [8].

Most actinobacteria are producers of valuable bioactive compounds; however, some actinobacterial genera, especially *Nocardia* species, can cause a disease called "nocardiosis" in patients with cell-mediated immunosuppressive conditions as well as in immunocompetent patients [9]. Some actinobacterial species, such as *Actinomadura madurae* and *Streptomyces somaliensis*, also cause mycetoma, a chronic granulomatous infection, presenting as subcutaneous tissue swelling of the affected area, nodule formation, and drainage through sinus tracts [10]. Consequently, susceptibility testing of

these actinobacterial pathogens will assist in disease treatment.

Sichang Island is the smallest district of Thailand and located in the Gulf of Thailand. The island is 12 km from the mainland of Chonburi Province and consists mainly of rocks, mountains, and crags. Soil from the island was expected to contain promising antibiotic producers and novel actinobacterial taxa.

This study focused on the isolation and antimicrobial activities screening of actinobacteria in soil samples collected from Sichang Island, Chonburi Province, Thailand. The antimicrobial susceptibilities of *Nocardia* isolates were also determined.

MATERIALS AND METHODS

Sample collection and isolation of actinobacteria

Soil samples were collected from six locations on Sichang Island, Chonburi Province, Thailand during the month of February 2017 (Fig. S1). The samples were preserved at 4 °C before transporting to the laboratory. *Actinobacteria* were isolated following the standard serial dilution methods using humic acid vitamin (HV) agar supplemented with nalidixic acid (50 μg/ml) and cycloheximide (25 μg/ml) [11]. The isolate plates were incubated at 30 °C for 14 days. Colonies of actinobacteria were observed under light microscope and selected for further purification on yeast extract-malt extract (ISP2) agar (yeast extract 4 g, malt extract 10 g, glucose 4 g, pH 7.0–7.2, added water up to 1 l). Pure cultures of actinobacteria were maintained on ISP2 agar at 30 °C.

16S rRNA gene and phylogenetic analyses

Actinobacterial DNA was extracted from mycelia, obtained from the culture grown in yeast-dextrose broth at 30 °C for 4–7 days, using a DNA extraction kit (Purelink™). The 16S rRNA gene amplification was carried out using primers 20F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1500R (5′-GTTACCTTGTTACGACTT-3′) [12]. The PCR products were purified using a Gel/PCR kit (Geneaid). Nucleotide sequencing of the PCR products was carried out using universal primers [13] (Macrogen, Seoul, Korea). BLASTN analysis of the 16S rRNA sequences was performed according to the EzBioCloud webpage (https://www.ezbiocloud.net) [14]. Sequences of all the actinobacterial isolates were aligned with selected sequences obtained from the GenBank/EMBLDDBJ database using BioEdit (Ibis Biosciences). A phylogenetic tree based on maximum likelihood was constructed using MEGA 7.0 [15] with all gaps eliminated before the calculation. The confidence values of tree nodes were evaluated using the bootstrap resampling method based on 1000 replications [16].

Antimicrobial screening

A cross-streak method was used to perform antimicrobial activity screening [17]. The actinobacteria were streaked on one side of the ISP2 agar plates and incubated at 30 °C for 14 days. Then, the tested microorganisms: *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 4341, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 were inoculated on the plates by a single streak perpendicular to the actinobacteria and incubated at 37 °C for 24 h. Finally, the inhibition area was recorded.

Antimicrobial susceptibility of Nocardia isolates

Susceptibility testing was performed by the Etest method using Ezy MIC™ strips (Himedia) on Mueller-Hinton (MH) agar plates inoculated by swabbing method. Minimum inhibitory concentrations (MICs) and resistance breakpoint were determined according to the Clinical and Laboratory Standards Institute (CLSI) criteria (M24-A2) [18]. All *Nocardia* isolates obtained were tested with ten antibiotics: amikacin, amoxicillin/clavulanate (2/1), ceftriaxone, cefotaxime, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, and trimethoprim/sulfamethoxazole. The MIC values were recorded after incubating the plates at 30 °C for 3 days.

RESULTS AND DISCUSSION

Identification and antimicrobial activity

Six rocky soil samples were collected from Sichang Island. Density of culturable actinobacteria in the soil samples ranged from 5×10^5 to 1×10^6 CFU/g, and 55 actinobacteria were isolated (Table 1). These bacteria were classified into three genus groups: *Streptomyces*, *Nocardia*, and *Saccharothrix* based on morphological characteristics and 16S rRNA gene analysis. The phylogenetic tree confirmed the classification into these three genera (Figs. 1 and 2).

Group I comprised 32 isolates (Fig. 1). These bacteria produced long-branching filamentous mycelia on the agar. Spiral chains and rectiflexibile type of spore chains were observed on the aerial

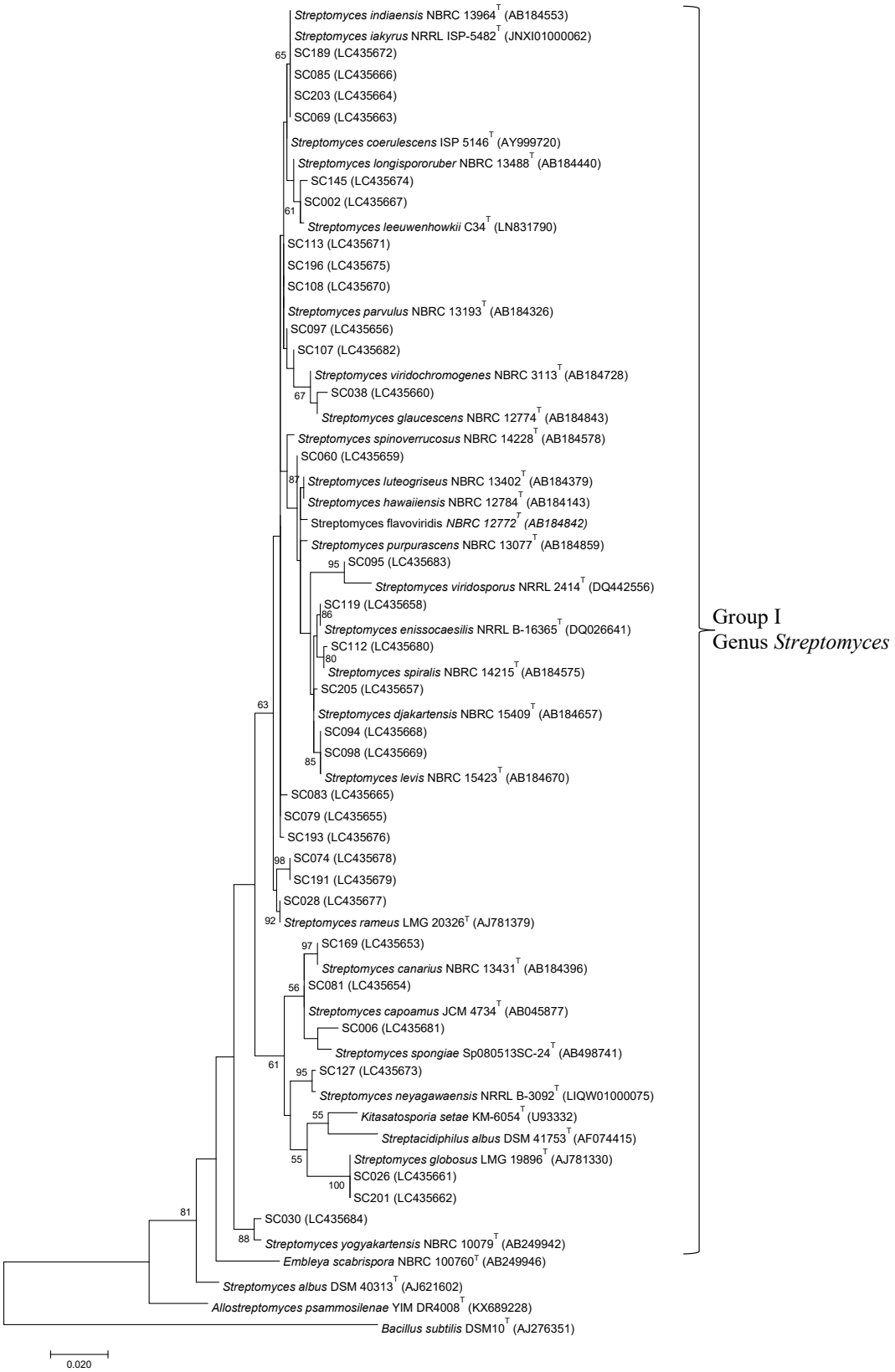

Fig. 1 Phylogenetic tree showing the relationship between actinobacterial isolates in group I and related actinobacterial species. *Bacillus subtilis* DSM10^{T}. was used as the outgroup.

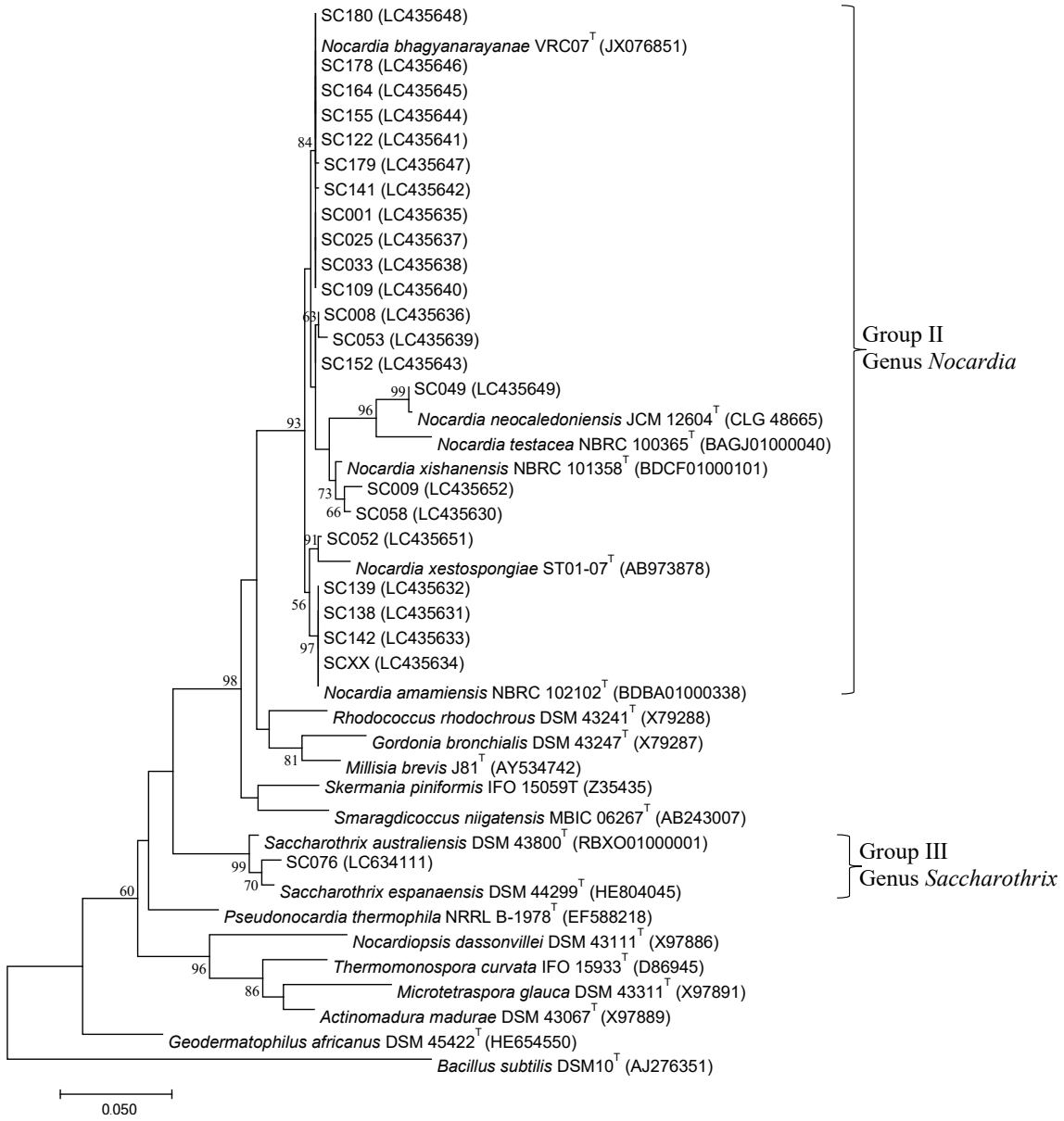

Fig. 2 Phylogenetic tree showing the relationship of the actinobacterial isolates in group II, group III, and related type strains. *Bacillus subtilis* DSM10^T was used as the outgroup.

mycelia. The representative isolate, SC095, produced a white to light gray aerial mass that could be differentiated from the spiral spore chain (Fig. S2). This spiral chain resembled characteristics of the genus *Streptomyces*. Based on the 16S rRNA gene analysis, these bacteria represented the highest similarity to those of the genus *Streptomyces*. Furthermore, members in this group were classified into 25 different closely related species (Table S1) of which 100% sequence similarity was observed with the 4 species: *S. enissocaesilis*, *S. globosus*, *S. levis*, and *S. rameus*.

Group II consisted of 22 isolates (Fig. 2). These bacteria produced white to pale cream aerial mycelia and pale orange substrate mycelia. Based on microscopic observation, branching filaments and fragmented zigzag conformation were observed on the aerial mycelia and substrate mycelia, respectively. The 22 strains did not produce any soluble pigment in the agar. The representative strain of this group, SC052, was also observed showing yellowish white aerial mass and grayish

Table 1 Number of actinobacterial isolates.

Sample no.	Isolate no.	Number of isolates
1	SC001, SC008, SC053, SC049, SC052, SC009, SC097, SC083, SC085, SC002, SC094, SC113, SC006, SC107, SC095, SC058	16
2	SCXX, SC141, SC081, SC079, SC145, SC074	6
3	SC060, SC076, SC152, SC155 SC069	5
4	SC138, SC139, SC025, SC033, SC109, SC122, SC119, SC038, SC026, SC098, SC108, SC127, SC028, SC112, SC030	15
5	SC142, SC164, SC178, SC179, SC180, SC196	6
6	SC169, SC203, SC205, SC189, SC193, SC191, SC201	7
	Total number of isolates	55

yellow substrate mycelia with fragmented substrate mycelia (Fig. S2). These characteristics were similar to members of the genus *Nocardia*. Based on the BLAST result of the 16S rRNA gene, actinobacteria in group 1 showed highest similarity with members of the genus *Nocardia*. These data were used to classify the actinobacteria in this group into six different closely related species as *Nocardia amamiensis*, *Nocardia bhayanarayanae*, *Nocardia lijiangensis*, *Nocardia neocaledoniensis*, *Nocardia xestospongiae* and *Nocardia xishaensis* (Table S1). Among these, isolates related to *N. bhayanarayanae* were the most frequent and five out of the six soil samples contained *N. bhayanarayanae* (Table 1).

Group III contained one isolate as SC076. This strain produced white aerial mycelia and pale-yellow green substrate mycelia. No pigment was observed on ISP3 agar. SC076 showed the closest 16S rRNA gene similarity (98.58%) to *Saccharothrix australiensis* DSM 43800^{T}.

In 2014, Kim et al suggested that a value of 98.65% 16S rRNA gene sequence similarity could be used as the threshold for differentiating two bacterial species [19]. In this study, three isolates including SC095, SC076, and SC052 showed 16S rRNA gene similarity lower than 98.65%, indicating that these three strains were candidates of novel actinobacterial taxa.

All the *Nocardia* isolates showed no antimicrobial activity against the tested microorganisms, while the 22 isolates of *Streptomyces* did. Besides, 21 *Streptomyces* isolates inhibited Gram-positive bacteria; but only three and seven isolates inhibited Gram-negative bacteria and yeast, respectively. Members of the *Streptomyces* genus that showed antimicrobial activity included *S. coerulescens* SC097, *S. djakartensis* SC205, *S. enissocaesilis* SC119, *S. globosus* SC201, *S. hawaiiensis* SC069, *S. iakyrus* SC203, *S. indiaensis* SC083, *S. levis* strains SC094 and SC098, *S. longispororuber* strains SC108 and SC113, *S. luteogriseus* SC189, *S. neyagawaensis* SC127, *S. parvulus* strains SC145 and SC196, *S. purpurascens* SC193, *S. spinoverrucosus* strains SC074 and SC191, *S. spiralis* SC112, *S. viridochromogenes* SC107, and *S. viridosporus* SC095. Interestingly, one candidate as the novel *Saccharothrix* SC076 showed potent antimicrobial activity against *K. rhizophila*, *B. subtilis*, *S. aureus*, *E. coli*, and *C. albicans*. Results in Table S1 indicated that strain SC076 showed broad-spectrum activity.

The *Streptomyces* genus is the largest antibiotic producer. However, since the 1970s, a number of antimicrobial compounds reported annually from this genus have substantially declined. *Streptomyces* is a common soil microorganism, and its isolation from soil over several decades leads to continued re-isolation of the same species. Consequently, obtaining novel compounds is difficult, and results often yield previously known compounds [20]. To solve this problem, several studies suggested that unexplored habitats offered promising sources of novel antibiotic producer. In 2017, Yun et al observed the diversity of soil actinobacteria collected from Ulleung Island, Korea. This Island was expected to yield unique microorganisms. They isolated 34 actinobacteria comprising *Streptomyces* (16 isolates), *Isoptericola* (5 isolates), *Rhodococcus* (4 isolates), *Agromyces* (3 isolates), *Micrococcus* (2 isolates), *Arthrobacter* (1 isolate), *Williamsia* (1 isolate), *Microbacterium* (1 isolate), and *Oerskovia* (1 isolate). Based on the phylogenetic tree, some of these actinobacteria represented candidates for novel species [21]. Later, in 2019, Sottorff et al analysed soil samples collected from Easter Island, Chile, and the samples comprised many novel candidate actinobacteria. A total of 163 actinobacterial isolates with 72 different phylotypes and 20 genera were found. The most abundant genera were *Micromonospora*, *Streptomyces*, *Salinispora*, and *Dietzia*. Interestingly, 45% of the actinobacteria from Easter Island showed a high degree of novelty as possible new taxa [22]. The results supported the

assumption that island soil harbors new actinobacterial species that might show a promise for further drug development.

Antimicrobial susceptibility of *Nocardia* isolates

The antimicrobial susceptibility of six closely related species of *Nocardia* including *N. amamiensis* ($n = 4$), *N. bhagyanarayanae* ($n = 11$), *N. neocaledoniensis* ($n = 1$), *N. xestospongiae* ($n = 1$), *N. lijiangensis* ($n = 1$), and *N. xishanensis* ($n = 1$) were determined using Etest. All isolates were found susceptible to imipenem and linezolid. The antimicrobial susceptibility profiles revealed that *N. amamiensis* was not susceptible to ciprofloxacin, ceftriaxone, and cefotaxime. Most strains of this species were resistant to clarithromycin (50%) and trimethoprim-sulfamethoxazole (75%) and susceptible to amikacin, minocycline, imipenem, linezolid, and amoxicillin/clavulanate (2/1). *N. bhagyanarayanae* was the most prevalent species obtained from Sichang Island. The results indicated that more than 90% of *N. bhagyanarayanae* was not susceptible to clarithromycin, ceftriaxone, cefotaxime, ciprofloxacin, and trimethoprim-sulfamethoxazole but susceptible to amikacin, linezolid and imipenem; while 27% were resistant to amikacin, and 73% showed intermediate resistant to minocycline.

Individual isolate of *N. neocaledoniensis*, *N. lijiangensis*, and *N. xishanensis* was obtained in this study. These strains were susceptible to amikacin, minocycline, imipenem, linezolid, and amoxycillin/clavulanate (2/1); except *N. xishanensis* ($n = 1$) that showed intermediate resistance to amoxycillin/clavulanate (2/1) (Table 2).

Strain SC052 showed a 97.8% similarity of the 16S rRNA gene. This strain should be a candidate of novel taxa. It was susceptible to amikacin, minocycline, imipenem, linezolid, and amoxicillin/clavulanate; but resistant to ciprofloxacin, ceftriaxone, cefotaxime, and trimethoprim-sulfamethoxazole (Table 2).

Nocardiosis is a common opportunistic infection found in immunocompromised patients. In 2005, Mootsikapun et al presented a review of nocardiosis cases from 1996 to 2001 in Srinagarind Hospital, Thailand. Data from 70 cases revealed that 80% of patients were male with mean age of 39.7 ± 14.9 years. The common diagnosis was a pleuropulmonary infection, followed by skin and soft tissue infection. In their study, 57.9% of the *Nocardia* isolates were resistant to trimethoprim-sulfamethoxazole [23]. Valdezate et al reported the antimicrobial susceptibility of *Nocardia* species in Spain. Most *Nocardia* strains were isolated from respiratory tract. They identified *N. cyriacigeorgica*, *N. nova*, *N. abscessus*, *N. farcinica*, *N. carnea*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. flavorosea*, *N. rhamnosiphila*, and *N. transvalensis* as active species against linezolid and amikacin [24]. In 2019, Lebeaux et al (in France) analyzed 793 *Nocardia* isolates collected between 2010 and 2015. These *Nocardia* were mainly isolated from lungs and comprised *N. farcinica*, *N. abcessus* complex, and *N. nova* complex. Active antibiotics against these species were linezolid, amikacin, trimethoprim-sulfamethoxazole, minocycline, and imipenem. *N. farcinica* showed a high rate (73%) of resistance to cefotaxime, while approximately 5% of *N. cyriacigeorgica* and *N. abscessus* were resistant to cefotaxime [25].

N. bhagyanarayanae and *N. amamiensis* were the most frequent species found on Sichang Island. Infection caused by *N. amamiensis* is rare, with only two ocular and two pulmonary infections previously reported [26, 27]. In 2016, Martinez-Gamboa et al reported pulmonary infection caused by *N. amamiensis* in Mexico. The strain was susceptible to trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid, imipenem, and amikacin [27]. Reddy et al reported two strains of *N. amamiensis* isolated from ocular infection [26]. Both strains were susceptible to tobramycin and amikacin but not susceptible to azithromycin and clarithromycin, while one isolate was not susceptible to gatifloxacin and ciprofloxacin. One strain of *N. bhagyanarayanae* was also reported for *Nocardia* keratitis [28].

N. neocaledoniensis is an uncommon cause of human infection and rarely documented for skin, soft tissue, and ocular infection [26, 29]. In 2020, the first fatal bacteremia case due to *N. neocaledoniensis* was reported [30], while an outbreak of *N. neocaledoniensis* mastitis in an Italian dairy herd was reported in 2008 [31]. At the time of reporting the present study, there has been no case report for both *N. lijiangensis* and *N. xishanensis* infections.

CONCLUSION

The most frequent taxa found as culturable soil actinobacteria of Sichang Island comprised the genera *Streptomyces* and *Nocardia*. Most *Streptomyces* strains showed antimicrobial activity. Three isolates from this study were identified as candidates of novel taxa. This number suggested that soil from the island was a promising source of novel acti-

Table 2 Antimicrobial susceptibility of *Nocardia* isolates to 10 antimicrobial agents.

Top-hit taxon based on 16S rRNA gene sequences	Isolate no.	MIC (μg/ml) (susceptibility)									
		CLA	CIP	AMK	MIN	IMI	CTX	LZD	AMC	CTR	TMP/SMX
N. amamiensis	SC138	6	>256 (R)	3 (S)	0.5 (S)	0.016 (S)	>256 (R)	1 (S)	4 (S)	>256 (R)	0.19 (S)
	SC139	3	>256 (R)	1 (S)	0.5 (S)	0.006 (S)	>256 (R)	1.5 (S)	6 (S)	>256 (R)	>32 (R)
	SC142	>256 (R)	8 (R)	4 (S)	0.5 (S)	0.016 (S)	>256 (R)	2 (S)	4 (S)	>256 (R)	>32 (R)
	SCXX	>256 (R)	>256 (R)	1 (S)	0.75 (S)	0.012 (S)	>256 (R)	1.5 (S)	4 (S)	>256 (R)	>32 (R)
N. bhagyanarayanae	SC001	>256 (R)	4 (R)	3 (S)	0.5 (S)	0.125 (S)	>256 (R)	2 (S)	1 (S)	>256 (R)	>32 (R)
	SC008	>256 (R)	8 (R)	16 (R)	2 (I)	0.032 (S)	>256 (R)	6 (S)	0.25 (S)	>256 (R)	>32 (R)
	SC025	6	8 (R)	8 (S)	2 (I)	0.125 (S)	>256 (R)	8 (S)	1.5 (S)	>256 (R)	>32 (R)
	SC033	>256 (R)	6 (R)	8 (S)	0.5 (S)	0.125 (S)	>256 (R)	1.5 (S)	0.75 (S)	>256 (R)	>32 (R)
	SC053	8 (R)	2 (I)	6 (S)	2 (I)	0.012 (S)	12	1 (S)	0.094 (S)	12	>32 (R)
	SC122	>256 (R)	8 (R)	24 (R)	2 (I)	0.125 (S)	>256 (R)	4 (S)	2 (S)	>256 (R)	>32 (R)
	SC141	8 (R)	8 (R)	4 (S)	0.75 (S)	0.094 (S)	>256 (R)	1.5 (S)	0.25 (S)	>256 (R)	>32 (R)
	SC152	0.38 (S)	4 (R)	12	2 (I)	0.047 (S)	>256 (R)	1 (S)	0.25 (S)	>256 (R)	>32 (R)
	SC164	>256 (R)	8 (R)	24 (R)	2 (I)	0.125 (S)	>256 (R)	1.5 (S)	0.75 (S)	>256 (R)	>32 (R)
	SC178	>256 (R)	8 (R)	8 (S)	2 (I)	0.094 (S)	>256 (R)	1.5 (S)	0.5 (S)	>256 (R)	>32 (R)
	SC179	16 (R)	8 (R)	4 (S)	0.38 (S)	0.125 (S)	>256 (R)	1 (S)	1 (S)	>256 (R)	>32 (R)
N. lijiangensis	SC058	>256 (R)	>256 (R)	8 (S)	1 (S)	0.094 (S)	>256 (R)	1.5 (S)	0.38 (S)	>256 (R)	>32 (R)
N. neocaledoniensis	SC049	>256 (R)	8 (R)	3 (S)	1 (S)	0.047 (S)	64 (R)	0.75 (S)	2 (S)	16 (I)	2 (S)
N. xestospongiae	SC052	6	2 (R)	3 (S)	2 (S)	0.38 (S)	>256 (R)	2 (S)	0.75 (S)	>256 (R)	>32 (R)
N. xishanensis	SC009	0.19 (S)	4 (R)	2 (S)	0.50 (S)	0.032 (S)	>256 (R)	2 (S)	16 (I)	>256 (R)	>32 (R)
Breakpoint (μg/ml)[a]	Susceptible (S)	⩽2	⩽1	⩽8	⩽1	⩽4	⩽8	⩽8	⩽8/4	⩽8	⩽2/38
	Intermediate (I)	4	2	–	2–4	8	13–32	–	16/8	16–32	–
	Resistant (R)	⩾8	⩾4	⩾16	⩾8	⩾16	⩾64	–	⩾32/16	⩾64	⩾4/76

[a]2011 M24-A2 CLSI interpretative criteria. CLA = clarithromycin; CIP = ciprofloxacin; AMK = amikacin; MIN = minocycline; IMI = imipenem; LZD = linezolid; AMC = amoxycillin/clavulanate (2/1); CTR = ceftriaxone; CTX = cefotaxime; and TMP/SMX = trimethoprim-sulfamethoxazole.

nobacteria. Many *Nocardia* species were isolated in this study, but these species are rarely reported as causing infection in humans. Antimicrobial susceptibility testing indicated that most *Nocardia* strains were susceptible to amoxicillin/clavulanate, imipenem, linezolid, and amikacin but not susceptible to ciprofloxacin, ceftriaxone, cefotaxime, and trimethoprim/sulfamethoxazole. However, the susceptibility profiles varied between strains and species.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2021.088.

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Appendix A. Supplementary data

Table S1 Closest BLASTN matches for the 16S rDNA sequence and antimicrobial activity of the actinobacterial isolates.

No.	Isolate no.	Top-hit taxon	Similarity (%)	Length (nt)	Accession no.	Antimicrobial activity[†]					
						S	B	K	E	P	C
1	SC138	*Nocardia amamiensis* NBRC 102102^{T}	99.93	1462	LC435631	–	–	–	–	–	–
2	SC139	*Nocardia amamiensis* NBRC 102102^{T}	100	1454	LC435632	–	–	–	–	–	–
3	SC142	*Nocardia amamiensis* NBRC 102102^{T}	99.35	1401	LC435633	–	–	–	–	–	–
4	SCXX	*Nocardia amamiensis* NBRC 102102^{T}	100	1457	LC435634	–	–	–	–	–	–
5	SC001	*Nocardia bhagyanarayanae* VRC07^{T}	99.44	1466	LC435635	–	–	–	–	–	–
6	SC008	*Nocardia bhagyanarayanae* VRC07^{T}	99.58	1460	LC435636	–	–	–	–	–	–
7	SC025	*Nocardia bhagyanarayanae* VRC07^{T}	100	1398	LC435637	–	–	–	–	–	–
8	SC033	*Nocardia bhagyanarayanae* VRC07^{T}	100	1400	LC435638	–	–	–	–	–	–
9	SC053	*Nocardia bhagyanarayanae* VRC07^{T}	99.44	1466	LC435639	–	–	–	–	–	–
10	SC109	*Nocardia bhagyanarayanae* VRC07^{T}	100	1414	LC435640	–	–	–	–	–	–
11	SC122	*Nocardia bhagyanarayanae* VRC07^{T}	100	1400	LC435641	–	–	–	–	–	–
12	SC141	*Nocardia bhagyanarayanae* VRC07^{T}	99.93	1394	LC435642	–	–	–	–	–	–
13	SC152	*Nocardia bhagyanarayanae* VRC07^{T}	99.72	1452	LC435643	–	–	–	–	–	–
14	SC155	*Nocardia bhagyanarayanae* VRC07^{T}	100	1396	LC435644	–	–	–	–	–	–
15	SC164	*Nocardia bhagyanarayanae* VRC07^{T}	99.79	1485	LC435645	–	–	–	–	–	–
16	SC178	*Nocardia bhagyanarayanae* VRC07^{T}	100	1427	LC435646	–	–	–	–	–	–
17	SC179	*Nocardia bhagyanarayanae* VRC07^{T}	99.90	1468	LC435647	–	–	–	–	–	–
18	SC180	*Nocardia bhagyanarayanae* VRC07^{T}	100	1463	LC435648	–	–	–	–	–	–
19	SC058	*Nocardia lijiangensis* NBRC 108240^{T}	99.75	834	LC435630	–	–	–	–	–	–
20	SC049	*Nocardia neocaledoniensis* JCM 12604^{T}	99.93	1388	LC435649	–	–	–	–	–	–
21	SC052	*Nocardia xestospongiae* ST01–07^{T}	97.84	1415	LC435651	–	–	–	–	–	–
22	SC009	*Nocardia xishanensis* NBRC 101358^{T}	99.17	1466	LC435652	–	–	–	–	–	–
23	SC169	*Streptomyces canarius* NBRC 13431^{T}	99.20	1461	LC435653	–	–	–	–	–	–
24	SC081	*Streptomyces capoamus* JCM 4734^{T}	99.65	1469	LC435654	–	–	–	–	–	–
25	SC079	*Streptomyces coerulescens* ISP 5146^{T}	99.77	1288	LC435655	–	–	–	–	–	–
26	SC097	*Streptomyces coerulescens* ISP 5146^{T}	99.44	1474	LC435656	±	±	+	–	–	–
27	SC205	*Streptomyces djakartensis* NBRC 15409^{T}	99.71	1423	LC435657	++	++	++			
28	SC119	*Streptomyces enissocaesilis* NRRL-B 16365^{T}	100	1469	LC435658	++	++	++	++	+	+
29	SC060	*Streptomyces flavoviridis* NBRC 12772^{T}	99.17	1471	LC435659	–	–	–	–	–	–
30	SC038	*Streptomyces glaucescens* NBRC 12774^{T}	99.61	1325	LC435660	–	–	–	–	–	–
31	SC026	*Streptomyces globosus* LMG 19896^{T}	100	1445	LC435661	–	–	–	–	–	–
32	SC201	*Streptomyces globosus* LMG 19896^{T}	100	1047	LC435662	–	±	–	–	–	–
33	SC069	*Streptomyces hawaiiensis* NBRC 12784^{T}	99.52	1471	LC435663	++	++	++	–	–	–
34	SC203	*Streptomyces iakyrus* NRRL ISP-5482^{T}	99.86	1402	LC435664	+	++	+	–	–	±
35	SC083	*Streptomyces indiaensis* NBRC 13964^{T}	99.31	1486	LC435665	++	++	++	–	–	+
36	SC085	*Streptomyces indiaensis* NBRC 13964^{T}	99.93	1473	LC435666	–	++	±	–	–	–
37	SC002	*Streptomyces leeuwenhoekii* C34^{T}	99.38	1461	LC435667	–	–	–	–	–	–
38	SC094	*Streptomyces levis* NBRC 15423^{T}	100	1410	LC435668	±	+	++	–	–	±
39	SC098	*Streptomyces levis* NBRC 15423^{T}	100	1459	LC435669	+	+	++	–	–	±
40	SC108	*Streptomyces longispororuber* NBRC 13488^{T}	99.44	1468	LC435670	–	–	–	–	±	–
41	SC113	*Streptomyces longispororuber* NBRC 13488^{T}	99.43	1452	LC435671	–	±	–	–	–	–
42	SC189	*Streptomyces luteogriseus* NBRC 13402^{T}	99.65	1457	LC435672	++	++	++	–	–	–
43	SC127	*Streptomyces neyagawaensis* NRRL-B 3092^{T}	99.59	1491	LC435673	++	++	++	–	–	–
44	SC145	*Streptomyces parvulus* NBRC 13193^{T}	98.96	1455	LC435674	–	±	–	–	–	++
45	SC196	*Streptomyces parvulus* NBRC 13193^{T}	99.86	1467	LC435675	++	++	++	–	–	–
46	SC193	*Streptomyces purpurascens* NBRC 13077^{T}	99.45	1472	LC435676	++	++	++	–	–	–
47	SC028	*Streptomyces rameus* LMG 20326^{T}	100	1412	LC435677	–	–	–	–	–	–
48	SC074	*Streptomyces spinoverrucosus* NBRC 14228^{T}	98.93	1428	LC435678	++	++	+	–	–	±
49	SC191	*Streptomyces spinoverrucosus* NBRC 14228^{T}	99.17	1482	LC435679	++	++	++	–	–	++
50	SC112	*Streptomyces spiralis* NBRC 14215^{T}	99.01	1122	LC435680	–	–	±	–	–	–
51	SC006	*Streptomyces spongiae* Sp080513SC-24^{T}	98.92	1393	LC435681	–	–	–	–	–	–
52	SC107	*Streptomyces viridochromogenes* NBRC 3113^{T}	99.36	1409	LC435682	–	–	++	–	±	–
53	SC095	*Streptomyces viridosporus* NRRL 2414^{T}	98.27	1487	LC435683	++	++	++	–	–	–
54	SC030	*Streptomyces yogyakartensis* NBRC100779^{T}	99.51	1432	LC435684	–	–	–	–	–	–
55	SC076	*Saccharothrix australiensis* DSM 43800^{T}	98.58	1414	LC634111	++	++	++	++	–	++

[*] Inhibition area: −, no inhibition area; ±, 1–5 mm; +, 6–10 mm; and ++, >10 mm.
Abbreviation: S, *Staphylococcus aureus*; B, *Bacillus subtilis*; K, *Kocuria rhizophila*; E, *Escherichia coli*; P, *Pseudomonas aeruginosa*; and C, *Candida albicans*.

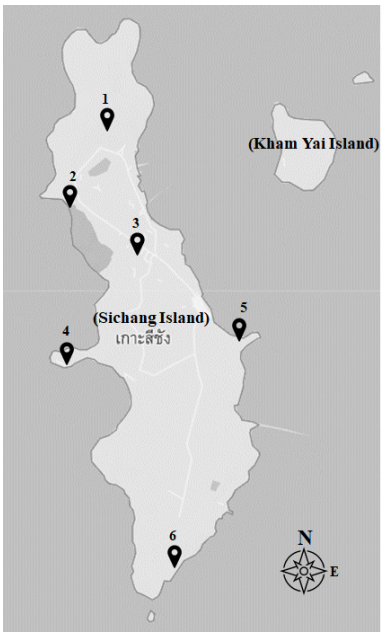

Fig. S1 Sampling site locations on Sichang Island, Chonburi Province, Thailand.

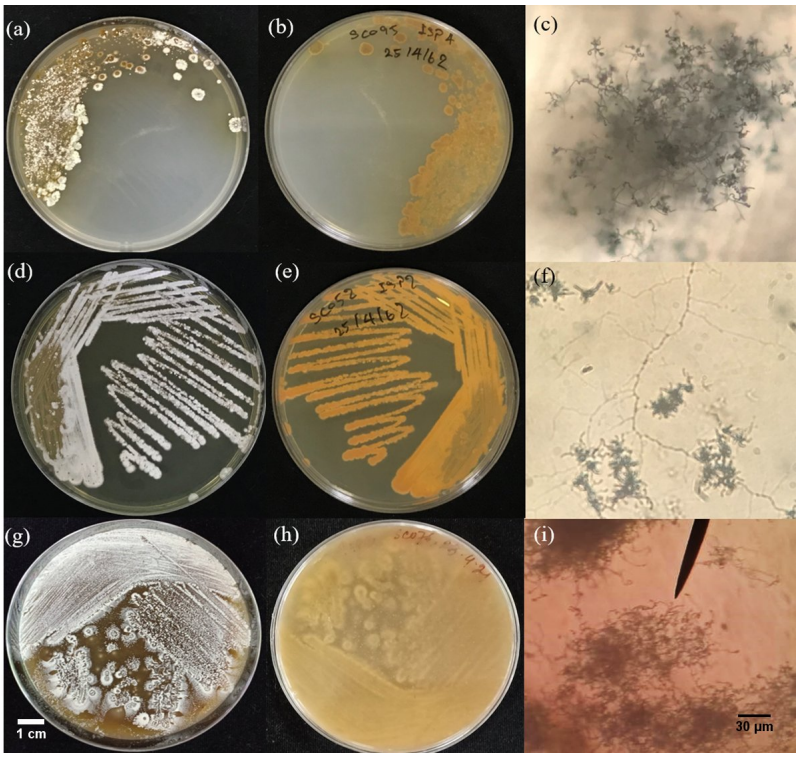

Fig. S2 Cultural characteristics and morphology of representative actinobacteria grown at 30 °C for 14 days: (a–c), *Streptomyces* sp. SC095 grown on ISP4 agar; (d–f), *Nocardia* sp. SC052 grown on ISP2 agar; and (g–i), *Saccharothrix* sp. SC076 grown on ISP2 agar. The white scale bar (1 cm) and the black scale bar (30 µm) indicate the size of the culture plates and microscopic pictures, respectively.