BIODIVERSITAS
Volume 22, Number 3, March 2021
Pages: 1104-1111

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220304

The potency of actinomycetes extracts isolated from Pramuka Island, Jakarta, Indonesia as antimicrobial agents

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Manuscript received: 5 May 2020. Revision accepted: 31 January 2021.

Abstract. *Setiawati S, Nuryastuti T, Sholikhak EN, Lisdiyanti P, Pratiwi SUT, Sulistiyanti TR, Ratnakomala S, Jumina, Mustofa. 2021. The potency of actinomycetes extracts isolated from Pramuka Island, Jakarta, Indonesia as antimicrobial agents. Biodiversitas 22: 1104-1111.* Actinomycetes are one of the Gram-positive bacteria which are widely distributed and produce many secondary metabolites including those known as antibiotics, antifungals, anticancer, and antimalarials agents. The secondary metabolites of actinomycetes are abundant, which include many active compounds that have been identified because of the large diversity in the actinomycetes phylum. This study aimed to identify and screen collected by Indonesian Culture Collection (InaCC) from Bojong Gede and Pramuka Island, Jakarta, Indonesia as antibacterial and antifungal agents. Primary screening was done on 16 actinomycetes isolates by well-agar diffusion method. Antimicrobial activity was tested by using micro broth dilution methods to determine minimum inhibitory concentration (MIC). Molecular identification into level genera and species was determined by 16S rRNA gene sequencing. Out of 16 actinomycetes isolates used, 4 isolates have activity against *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* BTCC B–612, and *Escherichia coli* BTCC B–614, specifically InaCC A758, InaCC A759, InaCC A760 and InaCC A765 isolates. InaCC A758 have highest antimicrobial activity against mentioned microbial with MIC value at 50 µg/mL, 6.25 µg/mL, 31.25 µg/mL and 3.125 µg/mL, respectively. Three genera were found from the samples: i.e. *Streptomyces* (80%), *Microbispora* (13%) and *Nocardia* (6%). Based on 16S rRNA gene identification, the active isolates of actinomycetes InaCC A758, InaCC A759, InaCC A760 and InaCC A765 were similar to *Streptomyces badius*, *Streptomyces olivaceus*, *Streptomyces sanyensis*, and *Nocardia otitidiscaviarum*, respectively. The secondary metabolites of actinomycetes extracts from Pramuka Island can be potentially developed as antifungal and antibacterial agents.

Keywords: 16S rRNA gene, actinomycetes, antibacterial, antifungal

INTRODUCTION

Actinomycetes are one of the Gram-positive bacteria that is widespread in various habitats and has been known to have many benefits as a source of drugs such as antibiotics, antifungal, antiviral, and anticancer agents (Ambavane et al. 2014; Balachandran et al. 2015; Barka et al. 2016). Secondary metabolites of actinomycetes are needed to protect themselves from pathogens in the environment. The production of secondary metabolites of actinomycetes is related to their life cycle and apoptosis process (Barka et al. 2016). Recently, most of the antibiotics known are produced by the genus *Streptomyces*.

Antimicrobial resistance has become a major global issue and will continue to have serious impact, especially increasing mortality and morbidity (Akova 2016). Many antifungal and anti-bacterial drugs were reported to be no longer effective because of developing resistance of pathogen. The emergence of azole resistance to *Candida* and *Aspergillus* species is a challenge in the treatment of systemic mycosis. The resistance rate of fluconazole to *Candida* species is diverse. *Candida krusei* has the highest global resistance rate compared to other species, which is 78.3% followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida albicans* (15.7%, 4.1%, 3.6% and 1.4%, respectively) (Sanguinetti et al. 2015). In several countries, the resistance rate of antifungal is various. In US, the resistance rate of *C. glabrata* to fluconazole reached 36% (Pham et al. 2014) and 14% in Kuwait (Sanglard 2016). The resistance rate of *C. albicans* to fluconazole in Mexico is very high up to 94.9% (Monroy-Pérez et al. 2016) followed in India (56.5%)

(Zaidi et al. 2018), Nepal 17.9%) (Khadka et al. 2017) and China (10.29%) (Maria et al. 2006). The resistance rate of *A. fumigatus* to azole was 27% in UK and 8 % in Netherlands (Sanglard 2016). In most of these cases, echinocandin was the only new class of antifungal drug used successfully in the last decade (Cui et al. 2015).

The management of infections related to methicillin-resistant *S. aureus* (MRSA) is still an extremely complicated problem. Multi-drug resistant (MDR) microbes found in hospital isolates are usually associated with methicillin resistance. Methicillin resistance rate in the US has gone up to 90% followed by levofloxacin (78.6%), ciprofloxacin (68%) and clindamycin (48.5%) (Akova 2016). *Escherichia coli* is a Gram-negative bacterium that produces extended-spectrum β-lactamases (ESBLs) which mostly cause bacteremia. ESBLs production is the main mechanism of *E. coli* resistance to penicillin and cephalosporins (Akova 2016). The resistance rate of *E.coli* to third-generation cephalosporins was 38.1% in Bulgaria and 11.9% in Europe (Hogberg et al. 2015). ESBL-producing *E. coli* are also resistant to non-β-lactamases (aminoglycosides and quinolone) (Kara et al. 2015). However, conventional antimicrobials are not useful against multidrug-resistance caused by Gram-positive and Gram-negative bacteria (Frieri et al. 2017). New strategies to find novel antifungal and antibacterial *Candida*tes that are safe, non-toxic, and more effective are direly needed.

Actinomycetes are found abundantly in the environment and have a large diversity of genera (Lisdiyanti et al. 2012; Widyastuti et al. 2012; Ratnakomala et al. 2016; Sulistyani et al. 2014). One of the actinomycetes habitats is the marine environment. At present, many researchers are focusing on the isolation of active compounds from marine actinomycetes. The secondary metabolites produced by actinomycetes include a wide range, i.e. polyene (Ambavane et al. 2014; Vartak et al. 2014), terpenoids (El-Sayed and Awad, 2013), phenolic (Belghit et al. 2016), polyketide (Oja et al. 2015; Asnani et al. 2016; Abdelmohsen et al. 2017), fenazin (Abdelmohsen et al. 2014), piperazin (Abdelmohsen et al. 2017), and non-polyene (Augustine et al. 2005). Many actinomycetes showing antimicrobial activities were found in marine habitats (Fenical and Jensen, 2006). They can be isolated from river flow, marine microbes associated with sponges, lake mud, river sediments, beach sands, marine sediments and mangrove forests (Eccleston 2008; Sunaryanto et al. 2012; Fadhilah et al. 2018).

Kepulauan Seribu has unique and specific ecosystem characteristics because it consists of sea waters with small islands around it. The area of Kepulauan Seribu is dominated by coral reef ecosystems, seagrass beds and mainland coral islands which were important habitats for various types of marine life. Pramuka Island, which is part of the Kepulauan Seribu, relatively has clear seawater and has marine life diversity (Sachoemar 2008). Fadhilah et al. (2018) had isolated 38 actinomycetes isolates from the mangrove ecosystem of Pramuka Island and found that 50% had antibacterial activities. Alfisyahri et al. (2018) also isolated 22 isolates of actinomycetes from sediment on the Pramuka Island. The results of screening for antibacterial activity showed that 13 isolates were able to inhibit *S. aureus* NBRC 100910 and 5 isolates were able to inhibit *C. albicans*. Based on the characteristic of the Pramuka Island, it is possible to find various species of actinomycetes that have active secondary metabolites. In this study, we identified the species of actinomycetes isolated from Bojong Gede and Kepulauan Seribu, Indonesia and investigated their secondary metabolites as antibacterial and antifungal agents.

MATERIALS AND METHODS

Sample site of actinomycetes isolation

Actinomycetes isolates used in this study were from the Indonesian Culture Collection (InaCC), Indonesian Institute of Science (LIPI) collections. Sixteen actinomycetes isolates were obtained from Bojong Gede, Bogor, West Java and Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia. Eight isolates were collected from rhizomes of *Curcuma zedoria*, four isolates from rhizospheres soil, two isolates from sediments in Pramuka Island and 2 isolates from sand beach in Pramuka Island.

Sixteen actinomycetes isolates were used in this study, i.e. InaCC A619, InaCC A621, InaCC A622, InaCC A623, InaCC A627, InaCC A633, InaCC A626, InaCC A641, InaCC A753, InaCC A75, InaCC A759, InaCC A760, InaCC A761, InaCC A765, InaCC A766 and InaCC A767. Actinomycetes isolates were grown in starch yeast pepton (SYP) agar medium and incubated at 30°C for 2-3 weeks (Lisdiyanti et al. 2011).

Secondary metabolites extraction of actinomycetes

Actinomycetes isolates were precultured in 10 mL SYP (starch 1%; yeast extract 0.4%; peptone 0.2%) liquid medium and incubated in a rotary shaker incubation (130 r/min) at 30°C for 3 days. The precultures were transferred into Erlenmeyer baffle flask (Choi et al. 2009) containing 90 mL SYP liquid medium (1:9) and incubated in a rotary shaker (130 r/min) at 30°C for 3 days.

One hundred milliliters of actinomycetes culture that had reached optimal growth in the SYP liquid medium were centrifuged at 3000 rpm for 15 min then the supernatant was added with 100 mL of ethyl acetate (1:1) and incubated for 12 h in a shaker at130 rpm. Then the solution was transferred into a separating funnel and the water phase (the bottom) was removed. The organic phase (upper phase) was transferred to the clean Corning tube and evaporated using a rotary evaporator at a pressure of 40 mPa at 40°C. The resulting dry extract (5 mg) was then dissolved into 5 mL DMSO 5% and used for bioassay.

Screening of antimicrobial activity

Sixteen fermented liquid samples of actinomycetes were tested against *C. albicans* ATCC 10231, *S. aureus* ATCC 6538, *Bacillus subtilis* BTCC B6-12, *P. aeruginosa* ATCC 27853, and *E. coli* BTCC B6-14 by well-agar diffusion method (Valgas et al. 2007). The bacterial inoculum was spread using sterile cotton swab on Muller Hinton (MH) agar and fungal inoculum was spread on

sabouraud dextrose agar (SDA). Fifty microliters of actinomycetes fermented liquid were added to each of the wells (7 mm diameter holes). The plates were inverted and incubated at 35°C for 24 hr. The antimicrobial activity was determined as clear inhibition zone around the wells. Inhibition of the microbial growth was measured in mm. The power of activity was classified based on inhibition zone diameters as strong (≥ 15.0 mm), moderate (10.0 to 14.5 mm) and weak (<10 mm) (Ahmad et al. 2005). Tests were performed in duplicate with three replicates.

Determination of minimum inhibitory concentration (MIC)

The MICs were determined using the microbroth dilution method described in the guidelines of the Clinical and Laboratory Standards Institute (CLSI) 2015 (CLSI, 2015). Briefly, serial two-fold dilutions were prepared in microplate 96-wells. One hundred microliters of bacterial cell suspension (~1x10^5 CFU/mL) or yeast cells suspension (~1x10^6 CFU/mL) which have been cultured for 24 h was added to each well. Negative (yeast or bacteria only) and positive (fluconazole or gentamycin) controls were included. The concentration of fluconazole and gentamycin was used at 50-0.196 µg/mL. The plates were incubated at 35°C for 24 h and the MIC values were determined by visual observation. Hazen (1998) defined that the MIC was the lowest concentration of drug that resulted in complete inhibition of growth of the fungi by visual observation. The growth inhibition of planktonic cells was determined by measuring the optical density at 595 nm. The percentage of inhibition was calculated using the following formula: Percentage inhibition = [(Control OD595-Treated OD595)/ (Control OD595-Blank OD595)] X 100 (Wang et al. 2017). All experiments were performed three times with three replicates for each experiment (Setiawati et al. 2017).

16S rRNA gene sequencing

Actinomycetes isolates were cultivated in Tryptic Soy Broth (TSB) (Oxoid, Britain) medium (5 mL) and incubated in a shaker incubator (120 rpm) overnight. The pellets were collected for extracting the genomic DNA using extraction buffer (200 mM Tris-HCl pH 8, 250 mM NaCl, 25 mM EDTA and 0.5% SDS) (Saito and Miura 1963). The polymerase chain reaction (PCR) primers were 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3') (Sulistyani et al. 2014). The PCR amplification used PCR Master Mix (Go Taq Green, Promega M7122) with total volume of 50 µL, consisting of 2 µL of each primer (20 pmol), 25 µL of master mix (Go Taq) PCR, 1 µL DNA template, and 20 µL dDH2O. PCR conditions were 96ºC for 5 min to pre-heating, then followed by 30 cycles at 96ºC for 30 seconds for denaturation, 55ºC for 30 seconds for annealing, 72ºC for 1 min for elongation, 72ºC for 7 min for primer extension (Sulistyani et al. 2014). After the cycle was complete, products continued cooling at 4ºC for 30 min. PCR reaction was conducted using a GeneAmp PCR System 9700 (Applied Biosystem Inc., Foster, California). The PCR products were examined by electrophoresis on agarose gel 1% to confirm that the target DNA had been amplified. The band formed could be expressed as a positive (+) result and compared with a marker to determine the target DNA band size of 1500 bp.

The purified PCR products were sequenced on an ABI 3730xl (Applied Biosystem Inc., Foster, California) DNA Sequencer with the BigDye Terminator version 3.1 cycle sequencing kit chemistry. The sequencing primer used 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3') (Sulistyani et al. 2014).

The 16S rRNA gene sequences were trimmed and assembled with BioEdit program. The nucleotide sequences' data of the isolates were compared with reference strains in the 16S rRNA gene database using BLAST (https://www.ezbiocloud.net/identify) and chosen based on a high similarity rank.

Construction and analysis of phylogenetic tree

The Molecular Evolutionary Genetic Analysis (MEGA) 7 software was used to generate evolutionary distances and similarity values. The phylogenetic tree was reconstructed by neighbor-joining (Saitou and Nei 1987) method. The phylogeny test was evaluated by bootstrap with 1000 replicates.

RESULTS AND DISCUSSION

Screening of antimicrobial activity of actinomycetes

To investigate antimicrobials potency, primary screening was performed by using fermented liquid of actinomycetes. The results of antifungal and antibacterial activity by well-agar diffusion method are shown in Table 1.

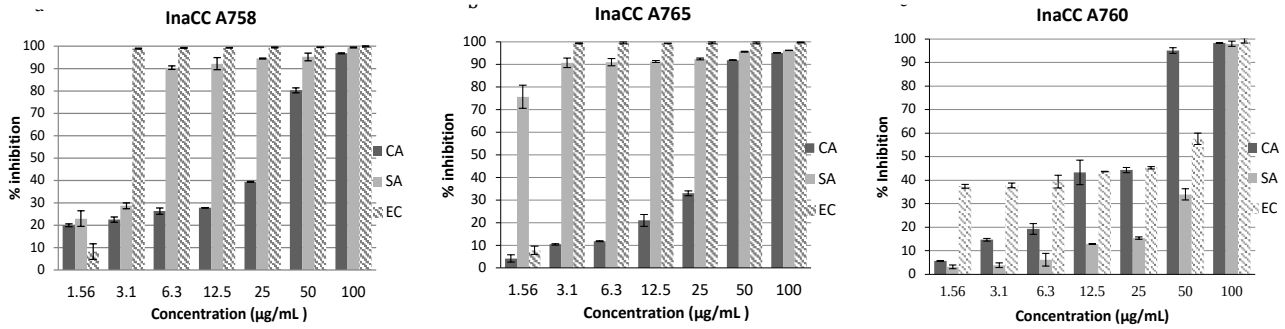
Among 16 actinomycetes tested, four isolates (InaCC A758, InaCC A759, InaCCA760, and InaCC A765) were active against *S. aureus* ATCC 6538, *B. subtilis* BTCC B-612, and *E. coli* BTCC B-614. None of the isolates was active against *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231. InaCC A758 isolates had the highest activity as antimicrobial against *S. aureus* ATCC 6538, *B. subtilis* BTCC B-612 and *E. coli* BTCC B-614 with the zones of inhibition were 28, 20 and 24 mm, respectively. Three active actinomycetes (InaCC A758, InaCC A759 and InaCC A760) were isolated from *Rhizospheres* soil and the others were isolated from sand beach in Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia.

Minimum inhibitory concentration (MIC)

The MIC values from ethyl acetate extracts produced by potential actinomycetes were observed (Table 2). Four of 16 extracts of actinomycetes had antifungal antibacterial activity (Table 1). InaCC A758 extracts inhibited fungus and all of bacteria tested, i.e *C. albicans* ATCC 10231, *S. aureus* ATCC 6538, *B. subtilis* BTCC B–612, *P. aeruginosa* ATCC 27853 and *E. coli* BTCC B–614 with the MIC values were 50, 6.25, 31.25, 500 and 3.125 µg/mL, respectively.

Inhibition percentages of actinomycetes extract against *C. albicans* ATCC 10231, *S. aureus* ATCC 6538 and *E.*

coli BTCC B–614 are shown in Figure 1. A758 crude extracts could inhibit *C. albicans* ATCC 10231, *S. aureus* ATCC 6538 and *E. coli* BTCC B 614 up to 80% at concentration 50 µg/mL, 6.3 µg/mL and 3.1 µg/mL, respectively. A 765 crude extracts could inhibit *C. albicans* ATCC 10231, *S. aureus* ATCC 6538 and *E. coli* BTCC B 614 up to 80% at concentration 50 µg/mL, 3.1 µg/mL and 3.1 µg/mL, respectively. A760 crude extracts could inhibit *C. albicans* ATCC 10231, *S. aureus* ATCC 6538 and *E. coli* BTCC B 614 up to 80% at concentration 50 µg/mL, 100 µg/mL and 100 µg/mL, respectively.



A **B** **C**

Figure 1. Inhibition percentage of actinomycetes extract against *C. albicans* ATCC 10231 (CA), *S. aureus* ATCC 6538 (SA), and *E. coli* (EC). A. Actinomycetes extract of InaCC A758 isolate; B. Actinomycetes extract of InaCC A765 isolate; C. Actinomycetes extract of InaCC A760 isolate.

Table 1. Antimicrobial activities of secondary metabolites produced by actinomycetes

Isolates	Geographical location	Sampling area	Zones of inhibition (mm)				
			CA	SA	BS	PA	EC
InaCC A 619	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 621	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 622	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 623	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 627	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 633	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 626	Bojong Gede, Bogor	Rhizome of *Curcuma zedoria*	-	-	-	-	-
InaCC A 641	Bojong Gede, Bogor	Stem of *Curcuma zedoria*	-	-	-	-	-
InaCC A 753	Pramuka Island	Sediment	-	-	-	-	-
InaCC A 758	Pramuka Island	Rhizosphere soil	-	28	20	-	24
InaCC A 759	Pramuka Island	Rhizosphere soil	-	6	13	-	14
InaCC A760	Pramuka Island	Rhizosphere soil	-	12	13	-	10
InaCC A 761	Pramuka Island	Rhizosphere soil	-	-	-	-	-
InaCC A 765	Pramuka Island	Sand beach	-	20	18	-	20
InaCC A 766	Pramuka Island	Sand beach	-	-	-	-	-
InaCC A 767	Pramuka Island	Sand beach	-	-	-	-	-
GEN*			-	20	22	12	14
5% DMSO			-	-	-	-	-

Note: CA: *C. albicans*; SA: *S. aureus*; BS: *B. subtilis*; PA: *P. aeruginosa*; EC: *E. coli*; GEN= gentamycin (10 µg/mL)

Table 2. The MIC values of ethyl acetate extracts of actinomycetes against fungi and bacteria

Extracts	MIC values (µg/mL)				
	C.albicans	***S.aureus***	***B.subtilis***	***P.aeruginosa***	***E.coli***
InaCC A758	50	6.25	31.25	500	3.125
InaCC A759	250	500	250	-	125
InaCC A760	50	100	-	-	100
InaCC A765	50	3.125	31.25	-	3.125
Fluconazole	1.56	-	-	-	-
Gentamycin	-	0.39	1.56	3.125	3.125

Identification of actinomycetes isolates based on 16S rRNA

The visualization of PCR using electrophoresis showed a band in the range of 1,500 bp for all actinomycetes isolates. This finding indicated that all isolates had a 16S rRNA gene marker. The results from BLAST analysis are shown in Table 3.

Three genera were found based on the phylogenetic tree (Figure 2) of 16 actinomycetes isolates, i.e *Streptomyces* (81%), *Microbispora* (13%) and *Nocardia* (6%). Based on 16S rRNA gene identification, the active isolates of InaCC A758, InaCC A759, InaCC A760 and InaCC A765 isolates are similar to *Streptomyces badius*, *Streptomyces olivaceus*, *Streptomyces sanyensis* and *Nocardia otitidiscaviarum*, respectively.

In this study, we used well-agar diffusion for screening of antimicrobial activity because this method has a good correlation to microdilution method, easy to reproduce, simple, inexpensive and easy to read (Magaldi et al 2004). The antimicrobial screening by well-agar diffusion method showed that the extracts of actinomycetes had activity against the *C. albicans* ATCC 10231 (Table 1) and surprisingly, it was contrary when tested by microbroth dilution method. There were 4 extracts that had activity against *C. albicans* ATCC 10231 (Table 2). In well-agar diffusion method, even fluconazole did not exhibit inhibitory activity against *C. albicans* ATCC 10231 (table 1). We assumed that the medium SDA that we used altered the diffusion process in agar medium. This finding linear with Magaldi et al. (2004) that reported the same result whereas the clear zone inhibition of fluconazole in well-agar diffusion method was diffused so it was difficult to observe and determine it. The Mueller-Hinton agar with 2% glucose and methylene blue was recommended in agar diffusion method as the clear zone can be seen clearly and the zone of inhibition can be determined easily (Magaldi et al. 2004).

In our study, there were 25% of actinomycetes extracts that demonstrated activity against *C. albicans* ATCC 10231 and broad-spectrum bacteria i.e. *S. aureus* ATCC 6538, *E. coli* BTCC B 614 and *B. subtilis* BTCC B 612 with moderate and strong activities. This results linear with Ryandin et al (2018) that reported the broad-spectrum antibacterial activity of crude extracts of actinomycetes isolated from Segara Anakan, Indonesia. They also used agar diffusion method and determined the zone of inhibition from these crude extracts against *E. coli*, *S. aureus*, *P. aeruginosa* and *Enterococcus* (12.5 mm; 21.5 mm; 10 mm; and 8 mm, respectively). Moreover, Arumugam et al (2017) reported the activity of crude extracts of actinomycetes isolated from soil samples of a mangrove forest, India exhibited fungal, Gram-positive and Gram-negative bacteria. On the contrary, Alfisyahri et al (2018) reported that crude extracts isolated from sediment in Pramuka Island, Indonesia detected no activity against Gram-negative bacteria. These differences in antimicrobial activity of actinomycetes can be altered the diversity of chemical structure, disintegration during the extraction process and environmental factors i.e. temperature and pH (Mohamed et al 2017).

Streptomyces InaCC A758, InaCC A759, InaCC A760 and InaCC A765 are actinomycetes isolated from marine habitats on Pramuka Island. Marine actinomycetes are known to have secondary metabolites that demonstrated potential to be antibacterial and antifungal agents. Mondol and Shin (Mondol and Shin 2014) isolated macrolactin compounds from marine sediments which inhibited Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) with MIC values in the range of 0.015-0.125 µg/mL. El-Sayed and Awad (2013) succeeded in isolating new compounds of bicyclic sesquiterpenoid derivatives from marine *Streptomyces* which have strong activity against Gram-positive bacteria, i.e *B. Subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. aureus* MRSA with MIC values 0.5, 1.5 and 1.3 µg/mL, respectively.

Table 3. The results of BLAST analysis using EzBioCloud database

Name of isolates	BLAST results	Length of 16S rRNA genes (bp)	Simi-larity (%)	Acc. no.
InaCC A619	*Streptomyces althioticus* strain NRRL B-3981 16S ribosomal RNA gene, partial sequence	1,405	99.36	MN826176
InaCC A621	*Streptomyces neopeptinius* strain KNF 2047 ribosomal RNA gene, partial sequence	1,413	98.92	MN826177
InaCC A622	*Streptomyces endophyticus* strain YIM 65642 16S ribosomal RNA gene, partial sequence	1,421	99.58	MN826178
InaCC A623	*Streptomyces griseorubiginosus* strain DSM 40469 16S ribosomal RNA gene, partial sequence	1,424	99.79	MN826179
InaCC A626	*Streptomyces griseorubiginosus* strain DSM 40469 16S ribosomal RNA gene, partial sequence	1,412	99.79	MN826180
InaCC A627	*Microbispora hainanensis* strain 211020 16S ribosomal RNA gene, partial sequence	1,357	98.44	MN826181
InaCC A633	*Streptomyces lannensis* strain TA4-8 16S ribosomal RNA gene, partial sequence	1,405	99.86	MN826182
InaCC A641	*Microbispora rosea subsp. rosea* strain ATCC 12950 16S ribosomal RNA gene, partial sequence	1,380	100	MN826183
InaCC A753	*Streptomyces parvus strain NBRC* 3388 16S ribosomal RNA gene, partial sequence	1,385	99.93	MN826184
InaCC A758	*Streptomyces badius* strain NRRL B-2567 16S ribosomal RNA gene, partial sequence	1,354	99.85	MN826185
InaCC A759	*Streptomyces olivaceus* strain B-3009 16S ribosomal RNA gene, partial sequence	1,407	99.93	MN826186
InaCC A760	*Streptomyces sanyensis* strain 219820 16S ribosomal RNA gene, partial sequence	1,414	99.58	MN826187
InaCC A761	*Streptomyces drozdowiczii* strain NBRC 101007 16S ribosomal RNA gene, partial sequence	1,411	99.50	MN826188
InaCC A765	*Nocardia otitidiscaviarum* strain NBRC 14405 16S ribosomal RNA gene, partial sequence	1,375	96.06	MN826189
InaCC A766	*Streptomyces zhihengii* strain YIM T102 16S ribosomal RNA gene, partial sequence	1,406	98.79	MN826190
InaCC A767	*Streptomyces iakyrus* strain NRRL ISP 5482 16S ribosomal RNA gene, partial sequence	1,418	99.08	MN826191

Figure 2

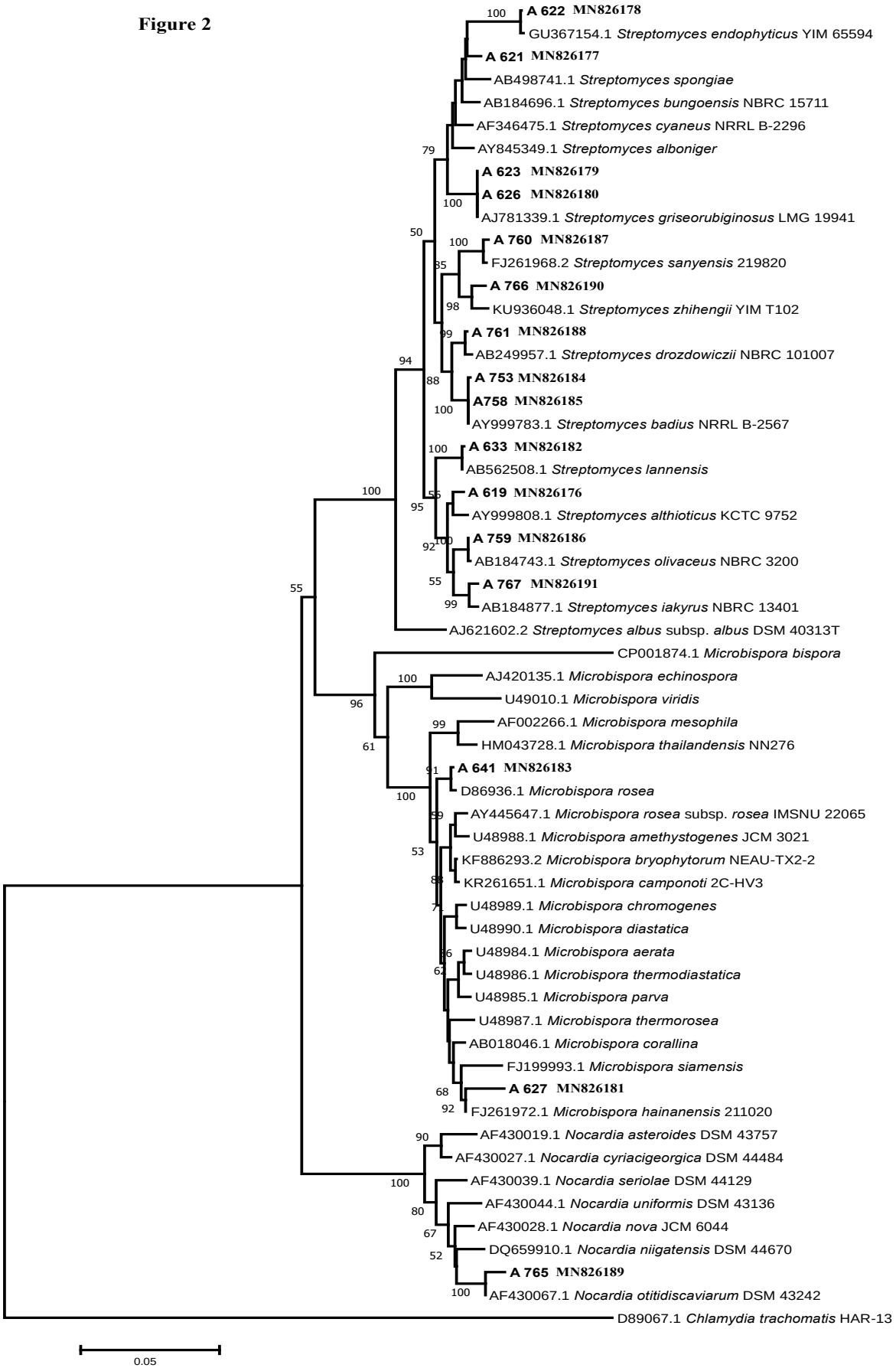


Figure 2. Phylogenetic tree of actinomycetes isolates based on partial sequences of 16S rRNA. The tree was constructed using the neighbor-joining method

Streptomyces are the predominant genus found in this study (80%). Seventy-five percent of active actinomycetes isolates in this study also come from *Streptomyces* species. This is not surprising because the genus *Streptomyces* is mostly found, especially in soil and produces the most secondary metabolites that are useful as antibacterial (50-55%) (Alharbi 2016). Saurav and Kannabiran (2012) also reported that the genus *Streptomyces* was the mostly genus discovered from soil samples in Uttarakhand, India. One of the active actinomycetes found in this study was identified as *Streptomyces badius* with a similarity of about 99.85%. Mohamed et al (2017) also reported that one of active actinomycetes isolated from Saharan soil in Algerian was identified as *S. badius* and had broad-spectrum antimicrobial activity. *Streptomyces* strain sourced from a different location will produce different secondary metabolites profile even though identification with 16S rRNA has similarities (Sottorff et al. 2019). Differences in the surrounding environment and competition for life over a long period of time cause the *Streptomyces* strain to acquire new abilities to produce different secondary metabolites. Sottorf et al (2019) examined two *Streptomyces* strains that had similarities based on 16S rRNA gene sequences but were isolated from different locations. The results showed that both *Streptomyces* strains had similarities in cell morphology although there were microscopical differences in pigmentation, air hyphae distribution and colony morphology, but both strains had a unique set of secondary metabolites for each isolate. Thus, there are still many opportunities to develop active compounds obtained from *Streptomyces*.

In our study, we also found active rare actinomycetes, i.e *Nocardia otitidiscaviarum*. Rare actinomycetes are usually found in less explored environments such as lake sediments, deep-sea, mangrove sediments, and marine sedimentation (Ramabhai and Mani-Jayaprakashvel 2019). Rare actinomycetes produce secondary metabolites which are believed to be able to provide promising results in the field of new drug discovery. Twenty-six percent of 10,000 metabolites isolated from actinomycetes actually were derived from rare actinomycetes (Bérdy 2005). Secondary metabolites produced by rare actinomycetes are very diverse and unique so they have very good bioactivity with low toxicity (Bérdy 2005).

The *Streptomyces* that was identified in this study has good activity as antifungal and broad-spectrum antibacterial. The limitation of this study is the small number of samples tested (16 isolates). Even though, it is has good potency to be developed as new drug *candida*te. Of course, it requires further study such as the activity against resistant microbial or against biofilm-forming microbial. It is also necessary to identify the active compound, mechanism of action and toxicity test of these *Streptomyces*. The purification and isolation of pure compound also needed for discovering the compound as drug *Candida*tes.

ACKNOWLEDGEMENTS

The experiment was performed in the Research Centre for Biotechnology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia and the Microbiology Department, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. We would like to give thanks to the Department for all facilities and to the superior basic research universities (PDUPT) grant from The Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding this research. The authors declare that there are no competing interests.

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