Antimicrobial Activities of Actinobacteria Isolated from Marine and Terrestrial Samples in Lombok Island, Indonesia

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Abstract

During our study on exploring actinobacteria from Indonesian sources, we selected 59 strains of actinobacteria isolated with five different isolation methods from Lombok Island Indonesia. The aim of this research was to isolate and characterize those actinobacteria, and to screen the microbial activities against 5 bacterial tested. Six soil samples from Lombok Island were processed. Totally, 59 isolated were selected. Of these strains, 31 strains showed antibiotic activity. Seventeen strains from 59 strains have been identified by 16S rRNA gene sequencing, and most dominant of the strains belongs to the genus *Streptomyces*. Three of them were chosen for further experiments due to their broad activity against four of five test strains. From two strains, at least one bioactive substance could be extracted. As only few bioactive substances could be extracted with ethyl acetate from liquid cultures more experiments should be performed on this subject. More solvent extraction should be tested as well as other production media. In conclusion, present study did now reveal bioactive actinobacteria were isolated from soil samples in Lombok Island. Antimicrobial compound has also been detected which is active against a broad range of pathogens. These findings may have an importance to produce potentially new antibiotic substances from actinobacteria indigenous Indonesia.

Keywords: actinobacteria, antimicrobial activity, Lombok Island Indonesia

Introduction

Antibiotics are drugs that are still being developed until today. Antibiotics can combat various infectious pathogens, reduce pain and suffering, revolutionize medicine, and increase human life span. Dias *et al*. (2012) and Demain and Zhang (2005) summarized that the challenging to develop new antibiotics are by discovering of new antibiotics from nature using high through put screening, modifying of a new semi-synthetic versions of old antibiotic, re-exploring of underutilized antibiotics, and developing of newly derivatives of previously undeveloped narrow-spectrum antibiotics using combinatorial biosynthetic and genome analysis. In term of discovering of new antibiotics from natural resources, marine and terrestrial ecosystems offer a bountiful supply of potentially novel microorganisms, which can be exploited in natural product screening programs (Tiwari & Gupta, 2012).

Actinobacteria are the most successful known antibiotic-producers, because they produce two thirds of all natural antibiotics use in clinic (Hug *et al*., 2018; Jagannathan *et al*., 2021). In the previous study, we have been isolated actinobacteria from several places in Indonesia and screen them for bioactive substances (Ratnakomala *et al*., 2016a, 2016b; Hatmanti *et al*., 2018; Putri *et al*., 2018; Susanti *et al*. 2019; Pahira *et al*., 2020). As Indonesia is one of the species-richest countries in the world (Myers, 1988), it is expected that this biodiversity is reflected in the microbiome and thus Indonesian soils are a source for unknown strains of actinobacteria,

which produce potentially new antibiotic substances.

Continuing of our studies, the aim of this study is to isolate actinobacteria from Lombok Island, Indonesia, screen them for antimicrobial activity against 5 bacterial tested, select the potential strains, identify to genus or species level by 16S rRNA gene sequencing. Previously, we have been isolated actinobacteria from Lombok Island (Lisdiyanti *et al*., 2012). We would like to compare those results.

Materials and Methods

Sample collection. The samples were collected from two different places in Lombok Island (West and North area of Lombok Island). In West area of Lombok Island (Cemara, Kuta, and Tanjung Kelor Beach), two samples from sea sands and two samples from mangrove sediment were collected (marine samples). In North area of Lombok Island (Genggelang Village), two samples from soils under cacao and coffee tree, two samples from leaf-litters under cacao tree, and two samples from peel of coffee bean were collected (terrestrial samples). All sampling time was conducted in April 2017. About 50 grams samples were taken and put on the plastic bags sterile and transported to the laboratory. These soil samples were air-dried for 34 h at 45°C, crushed, and sieved prior to use for isolation following established methods, except for dilution isolation method (Lisdiyanti *et al*., 2012).

Isolation methods. Isolation and enumeration of actinobacteria were performed on five different methods as follows:

(i) Dilution isolation method. The sample was serial diluted with sterile water to 10^{-3} and 10^{-4} and then 200 µL of solution from 10^{-3} and 10^{-4} were spread on HV agar plates (Dilution Isolation Method A) and HV agar plates with chlorine 1% (Dilution Isolation Method B). The plates were incubated at 30°C for 2-3 weeks. The composition of HV agar medium pH 7.2 was 1 % humic acid, 0.002 % $CaCO_3$, 0.001 % $FeSO_4.7H_2O$, 0.171 % KCl, 0.005 % $MgSO_4.7H_2O$, 0.05 % Na_2HPO_4, 0.0005 % cycloheximide, 1.8 % agar, and added with 1 ml B-vitamins after sterilization in 1 L medium. Dissolved humic acid was made by dissolved 1.0 g of humic acid in 0.4% of NaOH for 30 min at 100°C. B-vitamins was prepared by dissolved 0.5 mg of thiamin-HCl, 0.5 mg of riboflavin, 0.5 mg of niacin, 0.5 mg of pyridoxin-HCl, 0.5 mg of inositol, 0.5 mg of Ca-pantothenate, 0.5 mg of p-aminobenzoic acid, and 0.25 mg of biotin in 100 mL dH_2O, sterilized by 0.45 µm filter, and stored in 4°C (Hayakawa, 2008).

(ii) Rehydration-Centrifugation method. The sample was pestled and then sifted in a 500 µm sieve. A 0.5 g of sample was added to 50 mL 0.01 mM phosphate buffer with 1/10 soil extract (0.5 kg of soil was collected and stirred with 0.5 L of water. The suspension was autoclaved, then filtered and autoclaved again for 15 min.) The mixture of samples was kept for 30 min and then carefully transferred 10 mL from the upper part in a centrifuge tube and centrifuged for 20 min at 3,000 g (10^{-1}). It had to rest for exactly 15 min then 2 mL from the upper part were transferred to a new centrifuge tube (10^{-2}). The solution was serial diluted with sterile water to 10^{-3} and 10^{-4}, then a portion of 200 µL of solution of 10^{-3} and 10^{-4} were spread on HV agar plates three replication and incubated at 30°C for 2-3 weeks (Hayakawa, 2008; Lisdiyanti *et al*., 2010).

(iii) Dry-Heat method. The dried sample was grinded and sifted it in 500 µm sieve. Afterwards it was dried at 100°C for 40 min. It was put in a desiccator for cool down and by using small spatula small amount of dried sample was spread on an HV agar medium three replication, and incubated at 30°C for 2-3 weeks (Hayakawa, 2008).

(iv) SDS Yeast Extract (SY) method. A 0.5 g of air-dried sample was mixed in a centrifuge tube with 5 mL of sterile water, vortexed for 30 s, and kept for 30 s to precipitate the debris of sample (10^{-1}). A portion of 0.5 mL of the supernatant was transferred into a sterilized centrifuge tube containing 4.5 mL sterile sodium dodecyl sulphate (SDS) and yeast extract (SY) solution in phosphate buffer (10^{-2}). The composition of SY solution was 0.05 % SDS and 6 % yeast extract dissolved in 50 mM phosphate buffer pH 7.0 (1 M K_2HPO_4 and 1 M KH_2PO_4 that adjust to pH 7.0. The SDS inhibited the germination of the spores of bacterial cells and yeast extract activated spore germination of actinobacteria. Then, the sample and solution were heated in a 40°C water bath for 20 min. In a serial dilution, a

portion of 0.5 mL of the solution was diluted three times with sterile water (10^{-3}, 10^{-4}, and 10^{-5}). Aliquots of 200 µL from 10^{-3}, 10^{-4}, and 10^{-5} solution were plated three replications on HV agar medium and incubated at 30°C for 2-3 weeks (Hayakawa & Nonomura, 1989; Widyastuti *et al*., 2012).

(v) Phenol-method. A 0.5 g of heat dried sample was mixed with 4.5 mL of sterile water and shaken for 2 min (10^{-1}). A portion of 0.5 mL of the solution was added to 4.5 mL of phenol-phosphate-buffer (1.5 % phenol in 5 mM phosphate buffer) and kept at 30°C for 30 min (10^{-2}). The solution was diluted in sterile water two series (10^{-3} and 10^{-4}). A portion of 0.2 mL from 10^{-3} and 10^{-4} thereof was plated three replications on HV plates and incubated at 30°C for 2-3 weeks (Kumar & Jadeja, 2016).

Bioactivity tests using agar plug diffusion method. The isolates were grown on SYP NaCl agar medium (1 % starch, 0.4 % yeast extract, 0.2 % peptone, 1.8 % agar, 2 % NaCl, 0.005 % cycloheximide) for 10 days and agar plugs were tested against four standard bacterial strains and a yeast (*Bacillus subtilis* BTCC B-612*, Micrococcus luteus* BTCC B-*, Staphylococcus aureus* BTCC B-611*, Escherichia coli* BTCC B-609 and *Candida albicans* BTCC Y-33). A bottom layer of 15 mL agar medium was poured and dried. The bottom layer was covered by a top layer consisting of 5 mL soft agar mixed with 0.1-0.5 v/v% of an overnight culture of a standardized inoculum of the tested microorganism. The composition of growth media for *B. subtilis M. luteus, S. aureus,* and *E. coli* was Mueller-Hinton Agar medium (0.2 % beef extract, 1.75 % casein hydrolysate, 0.15 % starch, 1.8 % agar, pH 7.4) and for *C. albican* was Sabouraud Agar medium (4 % dextrose, 1 % peptone, 1.8 % agar, pH 5.6). Then, agar plug (about 6 mm in diameter), containing the test actinobacteria were placed on the agar surface. Agar plug were stanced from at least 10 days old SYP NaCl agar plates of the isolates and put on the test-plates. The Petri dishes were incubated at 30°C for overnight and the zone of inhibition was measured. Generally, antimicrobial agent diffused into the agar and inhibited germination and growth of the test microorganism and then the diameters of inhibition growth zones were measured (Pahira *et al*., 2000).

Determination of 16S rRNA gene sequence. Genomic DNA was extracted as described by Saito & Miura (1963) and 16S rRNA genes was amplified and sequenced according to Pahira *et al*. (2020). The 16S rRNA gene sequences determined in this study were manually aligned using BLAST with the published sequences of reference strains available from the EMBL/GenBank/DDBJ databases (Altschul *et al*., 1990).

Extraction of bioactive compounds. Hundred mL of culture medium in 500 mL flask was used for extraction bioactive compounds of actinobacteria. The medium was inoculated by 1 mL preculture and growth for 3 days at 30°C. After that, 7 mL of the culture were mixed with same volume of ethyl acetate. The mixture was shaken for 30 min and then centrifuged for 10 min at 3,000 rpm and room temperature. The organic phase was transferred into flask and evaporated at 40°C and 30 mbar. The residue was dissolved in 500 µL isopropanol to be used in bioactivity tests. Three different growing liquid media used in this study was ISP-2, NL-19 and NL-410. The composition of ISP-2 was 0.4 % yeast extract powder, 1.0 % malt extract powder, 0.4 % dextrose, and adjusted pH 7.3. The composition of NL-19 medium was 2.0 % soy flour, 2.0 % D-mannitol, and adjusted pH 7.2. The composition of NL410 medium was 1.0% glucose, 1.0 % glycerol, 0.5 % oatmeal, 1.0 % soy flour, 0.5 % yeast extract, 0.5 % Bacto casamino acids, 0.1 % $CaCO_3$, and pH was adjusted to 7.0 with NaOH.

Results

Isolation of actinobacteria.

Totally, 59 isolates were obtained on this study by using 6 different isolation methods, various kinds of samples in two sampling areas of Lombok Island. Of the 59 isolates, 25 isolates were obtained from sea sands and mangrove sediments in West area of Lombok Island (marine samples) and 34 origins from cacao- and coffee-leaf litter samples in North area of Lombok Island (terrestrial samples) (Table 1).

For samples from West area of Lombok Island (marine samples), 13 isolates were isolated by Dilution Isolation Method A (DA), 12 isolates by Dilution Isolation Method B

(DB), and no isolate was isolated by using RC method. Further, 8 samples were found from sea sand and 17 from mangrove sediment samples (Table 1).

For samples from North area of Lombok Island (terrestrial samples), 19 isolates were isolated by Dry Heat Isolation Method (DH), 12 isolates by SY Isolation Method, and 3 isolates by Phenol Isolation Method (PH). Further, 9 isolates were found from soil, 11 isolates from leaf litter, and 14 isolates from peel of coffee bean (Table 1).

Bioactivity tests using agar plate.

All 59 isolates were tested to preliminary antimicrobial assay by double layer agar block/plug method, on their bioactivity against *E. coli, B. subtilis, S. aureus, M. luteus* and *C. albicans.* Thirty-one (31) strains showed bioactivity against at least one of the five test strains (Table 2). Three strains exhibited 4 microbial tests *M. luteus, B. subtilis, E. coli,* and *S. aureus*, 1 strain exhibited 4 microbial tests *C. albicans, B. subtilis, E. coli,* and *S. aureus*, 13 strains exhibited 3 microbial tests *B. subtilis, E. coli,* and *S. aureus*, 3 strain exhibited 3 microbial tests *M. luteus, B. subtilis*, and *E. coli*, 2 strains exhibited 3 microbial tests *M. luteus, B. subtilis*, and *S. aureus*, 1 strain exhibited 2 microbial tests *B. subtilis* and *S. aureus*, 1 strain exhibited 2 microbial tests *E. coli* and *S. aureus*, 2 strains exhibited 2 microbial tests *M. luteus* and *B. subtilis*, 4 strains exhibited 1 microbial test *B. subtilis*, and 1 strain exhibited 1 microbial test *M. luteus,* (Table 2). Twenty-eight isolates did not have bioactivity against microbial testes.

Table 1. Source and Number of isolates

Sampling Area	Isolation Method	Source and Number of Isolates					Total Isolates
		Sea sand (2 samples)	Mangrove Sediment (2 samples)	Soil under cacao/ coffee tree (2 samples)	Litter under cacao tree (2 samples)	Peel of coffee bean (2 samples)	
West Lombok Island (Marine Samples)	Dilution Isolation Method A (DA)	8	5				**13**
	Dilution Isolation Method B (DB)	0	12				**12**
	Rehydration and Centrifugation Method (RC)	0	0				**0**
North Lombok Island (Terrestrial Samples	Dry Heat Isolation Method (DH)			3	8	8	**19**
	SDS Yeast extract Isolation Method (SY)			4	3	5	**12**
	Phenol Isolation Method (PH)			2	0	1	**3**
Total		**8**	**17**	**9**	**11**	**14**	**59**

Table 2. Isolated strains indicating the method of isolation, place of sampling and the size of the zones of inhibition in mm caused by agar block on various test bacteria.

No.	Code of Isolate	Source	Zones of inhibition in mm				
			Ca	*Ml*	*Bs*	*Ec*	*Sa*
1	DA-1 (I1)	Sea sands from Pantai Cemara, Lembar, West Lombok	-	14	23	30	31
2	DA-2	Sea sands from Pantai Cemara, Lembar, West Lombok	-	-	9.5	-	11.5
3	DA-3	Sea sands from Pantai Kuta, Lombok	-	-	14.5	16	12
4	DA-4	Sea sands from Pantai Kuta, Lombok	-	-	-	-	-
5	DA-5 (I2)	Sea sands from Pantai Kuta, Lombok	-	-	20	13	21
6	DA-6	Sediment mangrove from Pantai Cemara, West Lombok	-	-	-	-	-
7	DA-7	Sediment mangrove from Pantai Cemara, West Lombok	-	-	-	-	-
8	DA-8 (I3)	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	11.5	26.5	26.5	31
9	DA-9	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
10	DB-10	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
11	DB-11	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
12	DB-14	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
13	DB-15	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	11	11.5	12.5
14	DB-16 (I4)	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	20.5	18.5	21.5
15	DB-17	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
16	DB-18 (I5)	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	9.5	-	20.5	17	19.5
17	DB-19 (I6)	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	14	15	17
18	DB-20 (I7)	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	19	15	20
19	DB-21	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
20	DA-22	Sediment mangrove from Pantai Cemara, West Lombok	-	-	12	14	17
21	DB-23	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-

22	DB-25	Sediment mangrove from Pantai Tanjung Kelor, West Lombok	-	-	-	-	-
23	DA-26 (I8)	Sea sands from Pantai Kuta, West Lombok	-	-	17.5	13	15
24	DA-27 (I9)	Sea sands from Pantai Kuta, West Lombok	-	-	19	14.5	16.5
25	DA-28	Sea sands from Pantai Kuta, West Lombok	-	-	-	-	-
26	DH-29	Soil under cacao tree, Genggelang Village, North Lombok	-	-	-	13.5	15.5
27	DH-30 (I10)	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	23	22	23.5
28	DH-31	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
29	DH-32	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
30	DH-33	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	18	17	15
31	DH-34	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	10	-	-
32	DH-35	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	24	20	25
33	DH-36	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
34	DH-37	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	15	17	18
35	DH-38	Soil under coffee tree, Genggelang Village, North Lombok	-	-	-	-	-
36	DH-39	Soil under coffee tree, Genggelang Village, North Lombok	-	-	-	-	-
37	DH-40	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
38	DH-41	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
39	DH-42	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
40	DH-43	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
41	DH-44	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
42	DH-45	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
43	DH-46	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
44	DH-47	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
45	SY-48	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
46	SY-49	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
47	SY-50	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	12.5	-	-
48	SY-51	Soil under coffee tree, Genggelang Village, North Lombok	-	-	16	-	-
49	SY-52	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	-	-	-	-
50	SY-53	Soil under cacao tree, Genggelang Village, North Lombok	-	-	14	-	-
51	SY-54	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	12	11	-	-
52	SY-55	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	12	11	12	-
53	SY-56	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	11	11	-	-
54	SY-57	Leaf litter under cacao tree, Genggelang Village, North Lombok	-	12	-	-	-
55	SY-58	Soil under coffee tree, Genggelang Village, North Lombok	-	11	12	-	10
56	SY-59	Soil under coffee tree, Genggelang Village, North Lombok	-	12	11	-	10
57	PH-60	Soil under coffee tree, Genggelang Village, North Lombok	-	12	13	12	10
58	PH-61	Soil under coffee tree, Genggelang Village, North Lombok	-	11	13	14	-
59	PH-62	Coffee peel under cacao tree, Genggelang Village, North Lombok	-	10	12	12.5	-

Note:
Ca: Candida albicans, Ml: Micrococcus luteus, Bs: Bacillus subtilis, Ec: Escherichia coli; Sa: Staphylococcus auerus
DA: Dilution Isolation Method A; DB: Dilution Isolation Method B; DH: Dry Heat Isolation Method; YS: Yeast SDS Isolation Method; PH: Phenol Isolation Method.

Molecular identification based on 16S rRNA gene sequencing.

Phylogenetic tree of 16 strains based on 16S rRNA gene sequencing compare to type strains of the genus *Streptomyces* are shown in Figure 1. The analysis of the phylogenetic tree was conducted by comparing the sequences with type strains of the genus and type strain of outgroup from database. The phylogenetic tree showed that 15 strains belong to the genus *Streptomyces* (Figure 1). *Allostreptomyces psammosilenae*, *Embleya scabrispora*, *Streptacidiphilus albus* and *Kitasatospora setae* are the closest genera to *Streptomyces*, while *Chloroflexus aurantiacus* was added as an outgroup. Four strains were identified to the species level and eleven strains were identified to the genus level.

Strain DA-9 isolated from sediment mangrove was closely related to *Streptomyces cinereoruber* subsp. *fructofermentans* with boostraps value 94%, while strain DB-11 isolated from same location was closely related with *Streptomyces tirandamycinicus* with boostraps value 72%. Strain DB-18 (I5) obtained from sediment mangrove from Pantai Tanjung Kelor, West Lombok and strain DA-26 (I8) obtained from sea sands from Pantai Kuta, West Lombok were closely related with *Streptomyces spongiicola* with bootstraps value 94% and 79% respectively. Then, eleven strains were identified as *Streptomyces* sp. with bootstraps value below than 70%.

One strain, SY-54 that isolated from leaf litter under cacao tree, Desa Genggelang, North Lombok, was identified by *Letzea* sp. with similarity 99% to *Lentzea xinjiangensis* R24 (DQ898283) (Figure 2).

Extraction of bioactive compounds

The three isolates with the broadest activity against 4 of the five test strains were selected

for extraction. All three strains were isolated without further treatment. Strain DA-1 (isolated from sea sand) and strain DA-8 (isolated from mangrove sediment) showed activity against *E. coli, B. subtilis, S. aureus,* and *M. luteus.* Strain DB-18 (isolated from mangrove sediment) is active against *E. coli, B. subtilis, S. aureus,* and *C. albicans* using the method of this study.

The three selected strains were grown in three different liquid media (ISP-2, NL-19, and NL-410) and extracted with ethyl acetate. From the cultures of DA-1 strain in ISP-2, one substance could be extracted, which is active against *E. coli, B. subtilis,* and *S. aureus*, but not against *M. luteus* like from the result using the agar block. Accordingly, DA-1 strain produces more than one bioactive substance. The extract of a culture of DA-1 strain in NL-19 showed at least activity against *B. subtilis* (Figure 3A).

From the cultures of DA-8 strain in NL-19 and NL-410 one substance bioactive against *B. subtilis* could be extracted (Figure 3B).

Figure 1. Phylogenetic tree of 15 strains based on 16S rRNA gene sequencing compare to type strains of the genus *Streptomyces*. A tree was constructed by using MEGA 7 software with Neighbour-Joining method and evolutionary distances using p-distance (NJ-p) with 1000 replicates. *Allostreptomyces psammosilenae*, *Embleya scabrispora*, *Streptacidiphilus albus* and *Kitasatospora setae* are the closest genera of *Streptomyces*. Species *Chloroflexus aurantiacus* was added as an outgroup. The bootstraps above 70% was showed.

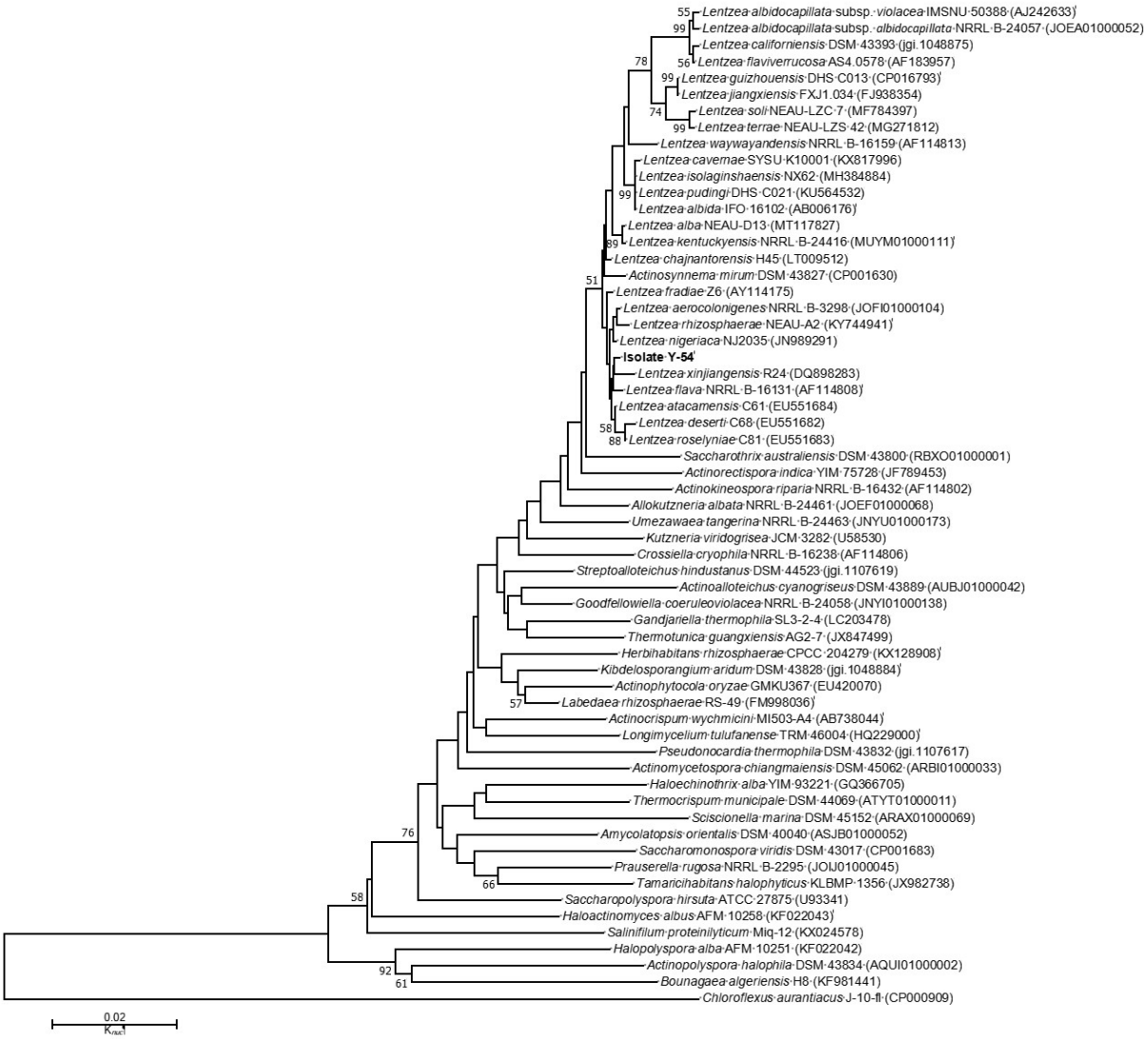

Figure 2. Phylogenetic tree of 1 strain based on 16S rRNA gene sequencing compare to type strains of the genus *Lentzea*. A tree was constructed by using MEGA 7 software with Neighbour-Joining method and evolutionary distances using p-distance (NJ-p) with 1000 replicates. *Allostreptomyces psammosilenae*, *Embleya scabrispora*, *Streptacidiphilus albus* and *Kitasatospora setae* are the closest genus of *Lentzea*. Species *Chloroflexus aurantiacus* was added as an outgroup. The bootstraps above 70% was showed.

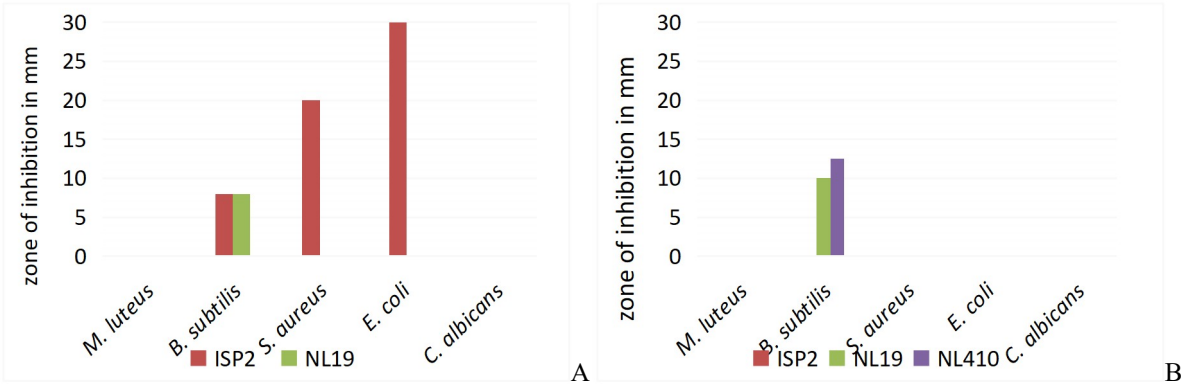

Figure 3. Zone of inhibition in mm caused extracts of DA-1 strain (A) and DA-8 on *M. luteus, B. subtilis, S. aureus, E. coli,* and *C. albicans* plates.

Discussion

Totally, 59 strains of actinobacteria could be isolated from Lombok Island using 5 isolation methods and using 2 sources. The 42 % was isolated from marine sources and 58 % was isolated from terrestrial sources. Fifty-two % of strains at least showed bioactivity against one of the five test strains, *E. coli, B. subtilis, S. aureus, M. luteus* and *C. albicans.*

Due to the limitation, only 27 % of the strains have been identified by 16S rRNA sequences. It should still be tested, by means of 16S rRNA sequencing, if with different methods the same strains were isolated and if the isolated strains are known already. Most of the strains could be isolated by not applying a special treatment. As among those there are the best antibiotic producers, these treatments are obsolete if only new antibiotics shall be found. This should be tested with a single sample on which all treatments are applied so the results can be compared.

As only few bioactive substances could be extracted with ethyl acetate from liquid cultures more experiments should be performed on this subject. Although Su *et al*. (2016), reported that the ethyl acetate extract from *Streptomyces* sp. P294 showed board spectrum activity against the tested bacterial pathogens. Valan *et al*. (2012), Lalitha & Gayathiri (2013), and Sengupta *et al*. (2015) had also reported that the ethyl acetate extract showed similar antibacterial activity. More extractants, for example ethanol, chloroform, n-hexan and butanol, should be tested as well as other production media.

In addition, an agar-extraction with water, acetone, and isopropanol should be performed, on the other hand to test polar solvents as extractors. The extraction of the antibiotics should be optimized and performed on a large scale, so enough material can be gained for structure analysis. To get a hint on the mode of action of the new antibiotics, the strains should be tested on resistance against established antibiotics, also to find possible markers for cloning experiments.

Lisdiyanti *et al*. (2012) reported that a total of 137 strains of actinobacteria were isolated from 14 soil samples collected in 3 parts of Lombok Island, Indonesia using 2 isolation methods (SDS-YE (SY) and Rehydration and Centrifugation (RC)) isolation methods and Humic Acid Vitamin (HV) agar as isolation media. The results of this study contributed the increasing of actinobacteria from Lombok Island that preserved in Indonesian Culture Collection (InaCC).

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