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IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF MARINE *Streptomyces* FROM GEOGRAPHICALLY DIFFERENT REGIONS OF INDONESIA

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Abstract

Identification and antimicrobial assay of indegenous marine *Streptomyces* have been conducted. Samples were obtained from culture collection of Biotech Center, Agency for the Assessment and Application of Technology (BPPT). They were originated from several Indonesian seashores including Pelabuhan Ratu, Pangandaran, Manokwari, Pulau Seribu, Garut, Bangka, Banjarmasin, Belitung, Cirebon, and Palu. Isolates stored as glycerol stocks were inoculated onto HV (Humic acid vitamin) agar and incubated for 5 days at 30 °C. Each colony was cultivated using yeast-peptone medium and then extracted by butanol. Antimicrobial activities were monitored by the agar diffusion paper-disc method against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 66923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* BIOMCC 00122 and *Aspergillus niger* BIOMCC 0013. Molecular identification of *Streptomyces* was carried out based on 16S rRNA gene analysis. Our research results showed that 71 isolates obtained from several Indonesian seashore were identified as 57 different *Streptomyces* species. Fifty of them showed antimicrobial activity. Twenty three isolates inhibited *B. subtilis* ATCC 66923, 14 isolates inhibited *S. aureus* ATCC 25923, 24 isolates inhibited *C. albicans* BIOMCC 00122 and 26 isolates inhibited *A. niger* BIOMCC 0013 and there was no active isolates inhibited the growth of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Phylogenetic analysis showed that *Streptomyces* isolates originated from the same geographically region was not necessarily grouped into the same cluster. Likewise a phylogenetic cluster may contain isolates of the same *Streptomyces* species, but from geographically different locations.

Keywords: isolation, identification, marine *Streptomyces*, Indonesian seashore

1. Introduction

Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. The marine environment may contain over 80% of world's plant and animal species (McCarthy & Pomponi, 2012). In recent years, many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and marine organisms (Ashawat et al., 2012). The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites, many of which are endowed with pharmacodynamic properties (Maithili et al., 2014).

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Streptomyces known as one of actinomycetes members that produce secondary metabolites, especially antibiotics (Blunt & Prinsep, 2006), anti-tumor and immunosuppressive (Berdy, 2005; Xu et al., 2014). *Streptomyces* have contributed to three-quarters of the discovered natural products and 80% of antibiotics reported so far (Bull & Stach, 2005; Kim & Garson, 2005). As many as 80% of secondary metabolites produced by *Streptomyces* was of *Streptomyces* spp. origin (Cragg & Newman, 2005).

Although the exploitation of marine *Streptomyces* as a rich source of pharmacologically important secondary metabolites is still at early stage, numerous novel metabolites have been isolated from this genus in the past few years. For example, oxoprothracarcin, a novel pyrrolo(1,4)benzodiazepine from marine *Streptomyces* sp. M10946 could provide new lead compounds for structural modification and drug screening (Han et al., 2013). Another example is the

novel alkaloid xinghaiamine A, an antibiotic with broad-spectrum antibacterial and cytotoxic activities from a marine *Streptomyces xinghaiensis* (Jiao et al., 2013). A novel anticancer 1, 2- benzene dicarboxylic acid, mono 2-ethylhexyl ester (DMEHE) from marine *Streptomyces* sp. VITSJK8 was found to be active against mouse embryonic fibroblast (NIH 3T3), human keratinocyte (HaCaT) normal cell lines, human hepatocellular liver carcinoma (HepG 2) and human breast adenocarcinoma (MCF-7) cell lines based on MTT assay (Krishnan et al., 2014).

Indonesia as an archipelago country with wide sea areas of more than 3.1 million km^2 is home to extremely high levels of biodiversity including a wide variety of microorganisms, plants, and animals (Hutomo & Moosa, 2005). Nevertheless the biotechnological potency of this marine biodiversity has not been optimally exploited. In particular, exploration of *Streptomyces* in Indonesia is currently still limited to terrestrial *Streptomyces*. Therefore, the objective of this research is to obtain insights into the biodiversity and antimicrobial activities of marine *Streptomyces* in Indonesian coastal waters.

2. Material and Methods

2.1. Isolation of Actinomycetes

All sediment samples were processed in laboratory immediately after collection. The samples were suspended in sterilized water and serial dilutions of each sample were made. The samples were individually treated with acid and heat-shock. Acid treatment was conducted by acidifying the samples to pH 2 for 3 hours. Heat-shock treatment was conducted by the heating the samples at 60 °C for 4 h (Pisano et al., 1986). The acid/heat-shock treated samples were then inoculated onto starch agar medium (1% w/v starch, 0.4% w/v yeast extract, 0.2% w/v peptone, natural seawater and 2% w/v agar) and incubated for 4-8 weeks at room temperature. One hundred gram per ml of nalidixic acid and 5 g/ml of rifampicin were added to reduce the number of unicellular bacteria (Pisano et al., 1989). The antifungal agent cycloheximide (100 g/ml) and 25 g/ml of nystatin were added to all isolation media. Actinomycetes colonies were recognized by the presence of branching, vegetative filaments and the formation of tough, leathery colonies that adhered to the agar surface. Morphologically diverse Actinomycetes were repeatedly transferred to the same media until pure cultures were obtained. All pure strains were grown in yeast extract-malt extract (YEME) broth and cryopreserved at -80°C in 10% v/v glycerol solution.

2.2. Preparation of Marine Streptomyces *Isolates*

One hundred marine *Streptommyces* isolates stored in glycerol stocks were cultured using HV (Humic acid vitamin) agar. Individual colonies that grew on such media were purified further by streaking them on new HV agar media, followed by incubation at 30°C for 5 days. Purified single colonies were selected for further study.

2.3. Identification of Streptomyces *using 16S rRNA*

Streptomyces were indentified by 16S rRNA gene analysis. The DNA was isolated using FastPrep kit (MP Biomedical) for DNA isolation. Briefly, the cell pellet was lysed using a lysing matrix, combined with 1 ml, and homogenized using a FastPrep instrument for 40 sec at 4500 rpm. PCR was performed using Forward primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-GGTTACC TTGTTACGACTT-3'), as described by Turner et al. (1999). PCR amplification was performed using GoTaq Green (Promega), 1 ml primer 8F and 1 ml primer 1492R, 20 ml DNA template. The final volume was 50 ml. PCR amplification was performed using the Takara Thermal Cycler. The program was as follows: 30 cycles 96 ºC for 2 min as a pre-denaturation step, 30 cycles of 96 ºC for 45 sec denaturation step, 30 cycles of 60 ºC for 20 sec annealing step, 30 cycles of 68 ºC for 1 min polymerization step, 68 ºC for 2 min post polymerization. The PCR products were visualized using gel electrophoresis on 1% agarose (Qiagen, Germany) and compared with 1 kb DNA ladder (Fermentas, Germany).

The PCR product was purified using a Gel/DNA extraction kit (Go Taq Green Mater Mix, Promega). The 16S rRNA gene obtained was submitted to the DNA sequencing facility, Genetic laboratory, Biotech Centre. A big Dye® terminator V 3.1 cycle sequencing kit was used to sequence the DNA. The DNA was then run in an automated DNA sequencer using capillary electrophoresis (ABI 300 genetic analyzer). The sequence was compared to a database available at NCBI using the BLAST search software (Altschul et al., 1990; McGinnis & Madden, 2004).

The homology of 16S rRNA sequences were search ed using Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) website (Altschul et al., 1990) and the reference sequences were retrieved from the GenBank (*www.ncbi.nlm.nih.gov*). Constructions of a phylo genetic tree was done using neighbour-joining tree method (NJT) implemented in MEGA 6.0 software (Tamura et al., 2011). Strength of internal branches of

the phylogenetic tree was tested with boostrap analysis using 1000 replications.

2.4. Liquid Medium for Production of Secondary Metabolite

An established agar slant of the *Stretomyces* isolate was inoculated into 50 ml of the vegetative medium Yeast Extract-Malt Extract (YEME) medium consisting of bacto peptone (5 g/L), yeast extract (3 g/L), malt extract (3 g/L), glucose (3 g/L), demineralized water (50 ml). The pH value of the medium was adjusted to 7.6 before sterilization. The YEME medium inoculated with such selected isolate was incubated at 30 ºC for 2 days in a shaking incubator. Five ml of the vegetative culture was transferred to 50 ml of the fermentation medium. The fermentation medium consists of glucose (4 g/L), bacto peptone (15 g/L), yeast extract (3 g/L), Fe (III) citrate hydrate (0.3 g/L), demineralised water (1000 ml) (Nedialkova & Mariana, 2005). The pH of the medium was adjusted to 7.6 before sterilization. The fermentation was carried out at 30 ºC for 5 days in incubator-shaker. Each treatment was fermented in triplicate.

2.5. Preparation of Extract Culture

Fifty ml of each culture broth was added with 50 ml of butanol and mixed thoroughly by shaking it for 30 min. Two layers were separated; and the organic layer was concentrated by evaporation under vacuum and added with 5 ml of methanol. Extracts were subjected to antimicrobial activity test.

2.6. Determination of Antimicrobial Activities

Antimicrobial activity was monitored by the agar diffusion paper-disc (6 mm) method. Discs were dripped with 15ml of the methanol extract (1 mg/ml), dried and then placed over the surface of the agar plates freshly inoculated with either *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *B. subtilis* ATCC 66923, *P. aeruginosa* ATCC 27853, *C. albicans* BIOMCC 00122 or *A.niger* BIOMCC 0013 as a test organism. Suspensions of such organisms were adjusted to 10^6 cfu/ml. Antimicrobial activity assay was conducted in triplicate. The antimicrobial activity was determined for each organism based on the mean diameter of inhibition zones according to the method of Bonev et al. (2008). Diameter of the inhibition zone was measured using a caliper micrometer gauge. Measurements were conducted from edge to edge across the zone of inhibition over the center of the disk.

3. Results and Discussion

The filamentous bacteria, especially *Streptomyces* are commonly found in all habitats. One hundred marine *Streptommyces* isolates stored in glycerol stocks were cultured in this study. Based on the growth of single colonies on an HV agar, 71 isolates were selected for further studies. The 16S rRNA gene analyses indicated that the seventy one *Streptomyces* isolates obtained in this study was categorized into 57 different *Streptomyces* species. Thirteen isolates originated from Manokwari, 10 isolates originated from Pelabuhan Ratu, 16 isolates originated from Pulau Seribu, 6 isolates from Banjarmasin, 6 isolates originated from Cirebon, 9 isolates originated from Garut, 3 isolates originated from Pangandaran, 3 isolates originated from Bangka, 4 isolates originated from Belitung, and 1 isolate originated from Palu. Three isolates were identified as *Streptomyces albidoflavus* spread in Manokwari and Pelabuhan Ratu. *Streptomyces albidoflavus were active against C. albicans* BIOMCC 00122 and *A. niger* BIOMCC 0013 (Tabel 1). *Streptomyces albidoflavus* is a Gram-positive, aerobic, filamentous actinomycete produced antifungal agents against plant pathogenic fungus *Rhizoctonia solani* AG2-2 (Islam et al., 2009) and *Passalora fulva* (Chen et al., 2015). *Streptomyces albidoflavus* have been used as potential biocontrol agent against the plant pathogenic fungus (Haggag et al., 2014).

Three isolates were identified as *Streptomyces coelicolor* found in Manokwari. This *Streptomyces coelicolor* were active against *C. albicans* BIOMCC 00122 and *A. niger* BIOMCC 0013. Leonid et al. (1996) reported that *Streptomyces coelicolor* strain isolated in Gottingen Germany produced blue pigment, named actinorhodin. *Streptomyces misionensis* was found in Banjarmasin and Belitung. These isolates were also active against *C. albicans* BIOMCC 00122 and *A. niger* BIOMCC 0013. *Streptomyces misionensis* was known to produce a thermoacidophilic endoglucanase (Cirigliano et al., 2013).

Streptomyces albogriseolus spread in Pulau Seribu and Bangka. This *Streptomyces albogriseolus* were active against *C. albicans* BIOMCC 00122 and *A. niger* BIOMCC 0013. *Streptomyces albogriseolus* HA10002 isolated from the mangrove sediment collected in Dongzhaigang Mangrove was known to produce antifungal and nematicidal compound fungichromin B (Qingfei et al., 2013). Two other isolates from Pulau Seribu were identified as *Streptomyces rochei*. These isolates *were also active against C. albicans* BIOMCC 00122 and *A. niger* BIOMCC 0013. *Streptomyces rochei* is a new potential biocontrol

Table 1. Bioactivity and identified isolate using 16S rRNA

	Sample code	Place of sample	(*) Identified isolate close related to	*B. subtilis* ATCC 66923		*E. coli* ATCC 25922		*S. aureus* ATCC 25923		*P. aeruginosa* ATCC 27853		*C. albicans* BIOMCC 00122		*A. niger* BIOMCC 0013	
				1	2	1	2	1	2	1	2	1	2	1	2
				mm		mm		mm		mm		mm		mm	
1	a 2	Manokwari	*Streptomyces macrosporeus* RA 16	-	-	-	-	-	-	-	-	14.91	14.85	9.23	8.97
2	a 5	Manokwari	*Streptomyces macrosporeus* RA 16	-	-	-	-	-	-	-	-	15.91	14.31	8.99	8.24
3	a6	Manokwari	Streptomyces flavoviridis AK1	-	-	-	-	-	-	-	-	-	-	-	-
4	a7	Manokwari	*Streptomyces californicus BI253*	-	-	-	-	-	-	-	-	-	-	-	-
5	a 9	Manokwari	Streptomyces coelicolor ST9	-	-	-	-	-	-	-	-	18.38	16.98	11.32	12.30
6	a10	Manokwari	Streptomyces coelicolor ST9	-	-	-	-	-	-	-	-	20.21	18.93	10.12	9.87
7	a 11	Manokwari	Streptomyces coelicolor ST9	-	-	-	-	-	-	-	-	20.81	19.32	11.76	12.32
8	a13	Manokwari	Streptomyces lienomyciniFoSt11	-	-	-	-	-	-	-	-	-	-	-	-
9	a15	Manokwari	*Streptomyces gramineus* FoRo24	-	-	-	-	-	-	-	-	-	-	-	-
10	a19	Manokwari	*Streptomyces carnosus* G614C1	17.14	15.80	-	-	-	-	-	-	-	-	-	-
11	a20	Manokwari	*Streptomyces carnosus* G614C1	19.60	16.83	-	-	-	-	-	-	-	-	-	-
12	a21	Manokwari	Streptomyces albidoflavus	-	-	-	-	-	-	-	-	21.63	20.17	12.68	11.68
13	a 22	Manokwari	*Streptomyces albidoflavus*	-	-	-	-	-	-	-	-	19.15	18.34	10.45	9.97
14	a23	Pelabuhan Ratu	*Streptomyces lavendulocolor* ANU020-vIB-101	24.23	25.10	-	-	-	-	-	-	-	-	-	-
15	a26	Pelabuhan Ratu	Streptomyces violascens RA 21	-	-	-	-	-	-	-	-	-	-	-	-
16	a 27	Pelabuhan Ratu	Streptomyces albidoflavus PAS-12	-	-	-	-	-	-	-	-	14.11	12.52	9.32	10.14
17	a 30	Pelabuhan Ratu	Streptomyces flavofungini Fole23	-	-	-	-	-	-	-	-	23.35	22.40	12.58	11.69
18	a33	Pelabuhan Ratu	*Streptomyces thermolineatus* 9-4	-	-	-	-	-	-	-	-	-	-	-	-
19	a 36	Pelabuhan Ratu	Streptomyces malaysiensis1160	-	-	-	-	11.73	11.54	-	-	17.95	17.16	13.80	15.39
20	a 38	Pelabuhan Ratu	Streptomyces flavofungini Fole23	-	-	-	-	-	-	-	-	21.39	20.74	12.36	11.48
21	a41	Pelabuhan Ratu	*Streptomyces coeruleofuscus*	24.11	22.32	-	-	-	-	-	-	-	-	-	-
22	a52	Pelabuhan Ratu	Streptomyces atratus strain 133	22.59	21.28	-	-	-	-	-	-	-	-	-	-
23	a 150	Pelabuhan Ratu	Streptomyces pactum strain EB60	22.64	20.31	-	-	-	-	-	-	-	-	-	-
24	a126	Pulau Seribu	Streptomyces ginsengisoli Gsoil 025	-	-	-	-	-	-	-	-	-	-	-	-
25	a 129	Pulau Seribu	Streptomyces narbonensis 0511ARD6F2	-	-	-	-	8.97	8.47	-	-	-	-	-	-
26	a 131	Pulau Seribu	Streptomyces parvus MJM10108	27.40	27.46	-	-	27.19	25.81	-	-	-	-	8.36	9.52
27	a 132	Pulau Seribu	*Streptomyces violaceorectus* RAM15B4	-	-	-	-	8.02	8.08	-	-	-	-	-	-
28	a 133	Pulau Seribu	*Streptomyces griseorubens* MMH 9	16.60	15.97	-	-	9.14	15.73	-	-	-	-	-	-
29	a 134	Pulau Seribu	Streptomyces termitum NRRL B-3804	12.32	11.54	-	-	9.34	9.65	-	-	-	-	-	-

Continued Table 1. Bioactivity and identified isolate using 16S rRNA

	Sample code	Place of sample	(*) Identified isolate close related to	*B. subtilis* ATCC 66923		*E. coli* ATCC 25922		*S. aureus* ATCC 25923		*P. aeruginosa* ATCC 27853		*C. albicans* BIOMCC 00122		*A. niger* BIOMCC 0013	
				1	2	1	2	1	2	1	2	1	2	1	2
				mm		mm		mm		mm		mm		mm	
30	a135	Pulau Seribu	*Streptomyces variabilis* STR61	9.31	9.87	-	-	-	-	-	-	-	-	-	-
31	a 136	Pulau Seribu	Streptomyces albogriseolus SCSAAB0028	-	-	-	-	-	-	-	-	11.08	9.83	9.32	8.35
32	a 137	Pulau Seribu	Streptomyces parvus MJM10108	34.44	30.82	-	-	25.87	25.35	-	-	-	-	8.72	8.52
33	a 138	Pulau Seribu	*Streptomyces bikiniensis* ZD_Hu	16.87	16.14	-	-	12.31	12.15	-	-	-	-	-	-
34	a139	Pulau Seribu	Streptomyces minutiscleroticus St62	-	-	-	-	-	-	-	-	-	-	-	-
35	a 140	Pulau Seribu	*Streptomyces rochei.* R1-2A/D104	-	-	-	-	-	-	-	-	13.88	11.07	9.25	9.23
36	a141	Pulau Seribu	Streptomyces geysiriensis 100	21.09	19.93	-	-	-	-	-	-	-	-	-	-
37	a142	Pulau Seribu	*Streptomyces labedae.* NBRC 15864	-	-	-	-	-	-	-	-	-	-	-	-
38	a143	Pulau Seribu	*Streptomyces rochei.* R1-2A/D104	-	-	-	-	-	-	-	-	14.12	12.75	8.28	9.41
39	a 152	Pulau Seribu	Streptomyces olivaceus FoRh5	19.64	14.98	-	-	-	-	-	-	-	-	-	-
40	a 83	Bangka	Streptomyces matensis St-5	-	-	-	-	-	-	-	-	8.96	10.81	8.58	8.02
41	a85	Bangka	*Streptomyces albogriseolus* NWU129	-	-	-	-	-	-	-	-	15.81	14.55	9.52	9.61
42	a 88	Bangka	Streptomyces erythrogriseus 7-6	13.05	10.43	-	-	12.03	11.55	-	-	-	-	-	-
43	a 92	Pangandaran	Streptomyces rishiriensis LuP30	-	-	-	-	-	-	-	-	9.09	12.55	10.70	8.42
44	a93	Pangandaran	*Streptomyces griseocarneus* 173667	12.28	10.34	-	-	-	-	-	-	-	-	-	-
45	a94	Pangandaran	*Streptomyces griseocarneus* 173931	20.65	17.96	-	-	-	-	-	-	-	-	-	-
46	a65	Banjarmasin	Streptomyces avermitilis CB2Z2	-	-	-	-	-	-	-	-	-	-	-	-
47	a66	Banjarmasin	Streptomyces novaecaesareae DSM 40358T	-	-	-	-	-	-	-	-	-	-	-	-
48	a 69	Banjarmasin	Streptomyces misionensis PESB 25	-	-	-	-	-	-	-	-	19.18	16.91	10.94	9.42
49	a73	Banjarmasin	*Streptomyces lunaelactis* MM109	-	-	-	-	-	-	-	-	-	-	-	-
50	a74	Banjarmasin	*Streptomyces lunaelactis* MM109	-	-	-	-	-	-	-	-	-	-	-	-
51	a 77	Banjarmasin	*Streptomyces somaliensis* SL4-1	-	-	-	-	-	-	-	-	21.63	20.17	12.68	9.65
52	a192	Belitung	Streptomyces costaricanus FoRh20	-	-	-	-	-	-	-	-	-	-	-	-
53	a 196	Belitung	Streptomyces misionensis JCM 4497	-	-	-	-	-	-	-	-	13.26	10.22	9.54	8.23
54	a 198	Belitung	*Streptomyces lannensis* FoRh3	-	-	-	-	-	-	-	-	17.35	17.21	9.95	8.10
55	a 199	Belitung	Streptomyces drozdowiczii OS-AC01	16.79	13.55	-	-	18.27	16.92	-	-	-	-	-	-
56	a156	Cirebon	*Streptomyces thermocarboxydus* DBT31	12.43	10.65	-	-	11.23	11.32	-	-	-	-	-	-
57	a157	Cirebon	Streptomyces bikiniensis JS-15	14.52	15.61	-	-	13.12	12.53	-	-	-	-	-	-
58	a158	Cirebon	Streptomyces beijiangensis TA13	14.38	13.63	-	-	17.97	15.98	-	-	-	-	-	-
59	a161	Cirebon	Streptomyces longispororuber TY0110	-	-	-	-	-	-	-	-	-	-	-	-

Continued Table 1. Bioactivity and identified isolate using 16S rRNA

	Sample code	Place of sample	(*) Identified isolate close related to	*B. subtilis* ATCC 66923		*E. coli* ATCC 25922		*S. aureus* ATCC 25923		*P. aeruginosa* ATCC 27853		*C. albicans* BIOMCC 00122		*A. niger* BIOMCC 0013	
				1	2	1	2	1	2	1	2	1	2	1	2
				mm		mm		mm		mm		mm		mm	
60	a 163	Cirebon	Streptomyces cavourensis A15	-	-	-	-	-	-	-	-	14.75	13.95	13.65	13.49
61	a164	Cirebon	*Streptomyces panayensis* 1043	-	-	-	-	-	-	-	-	-	-	-	-
62	a181	Garut	Streptomyces glomeratus NBRC 15898	-	-	-	-	-	-	-	-	-	-	-	-
63	a 182	Garut	*Streptomyces galbus* DSM 40089	-	-	-	-	9.21	8.25	-	-	-	-	-	-
64	a183	Garut	*Streptomyces hygroscopicus* FoRh26	-	-	-	-	-	-	-	-	-	-	-	-
65	a184	Garut	*Streptomyces glauciniger* D501	-	-	-	-	-	-	-	-	-	-	-	-
66	a185	Garut	Streptomyces pratensis XJB-YJ17	18.70	15.21	-	-	-	-	-	-	-	-	-	-
67	a187	Garut	Streptomyces cyanoalbus NBRC 12857	-	-	-	-	-	-	-	-	-	-	-	-
68	a188	Garut	Streptomyces coelicoflavus GLY-20	-	-	-	-	-	-	-	-	-	-	-	-
69	a 189	Garut	Streptomyces intermedius NBRC 13049	-	-	-	-	-	-	-	-	16.10	17.00	11.69	9.71
70	a 190	Garut	Streptomyces cavourensis A15	-	-	-	-	-	-	-	-	15.36	14.85	17.64	16.10
71	a103	Palu	Streptomyces echinatus NBRC 12763	19.80	19.17	-	-	-	-	-	-	-	-	-	-

Explanation: (*) partial nucleotide sequence shown in supplementary section

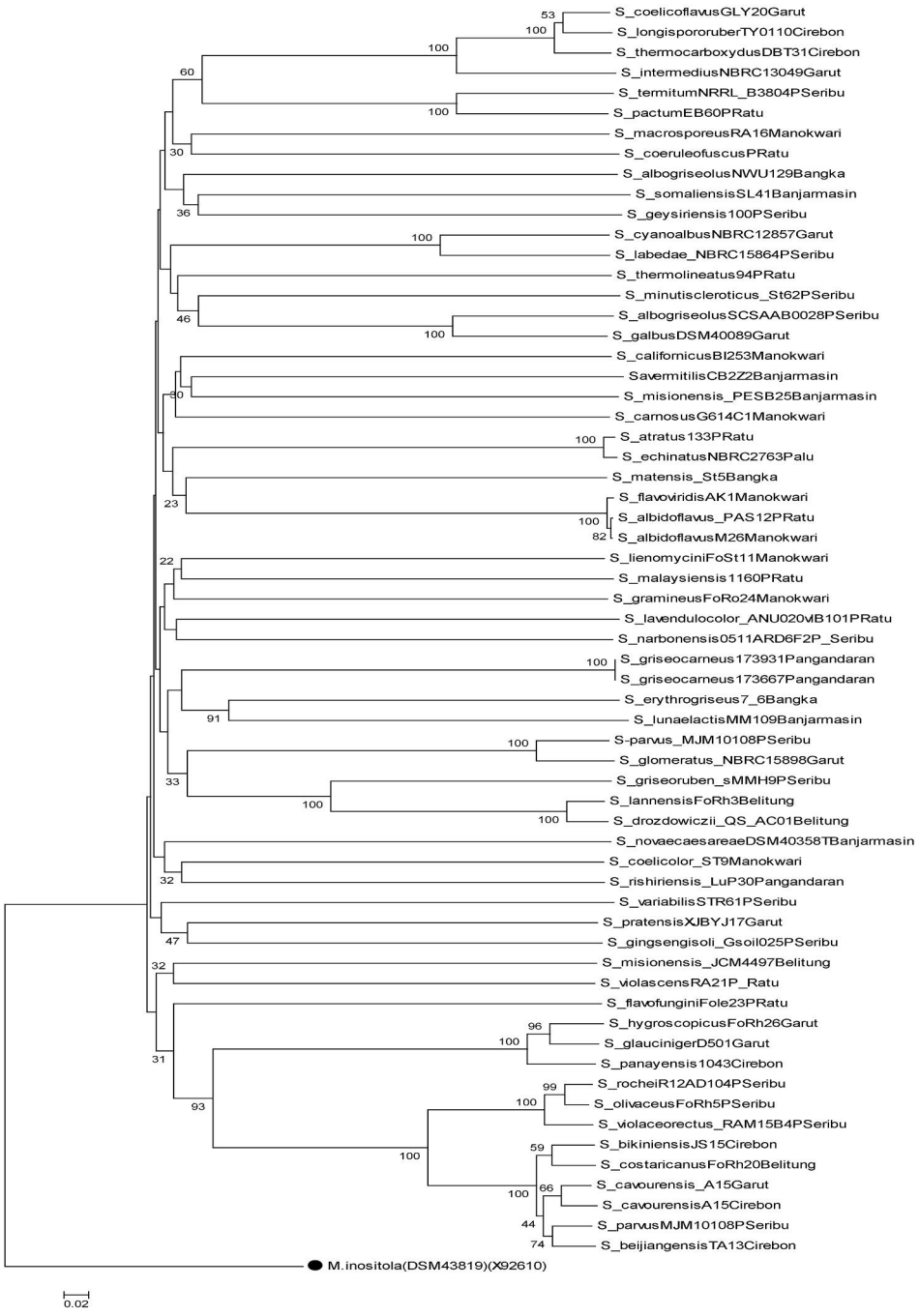

Figure 1. Phylogenetic tree constructed using neighbor-joining method with the aid of MEGA 6.0 program. The numbers at the branching points are the percentages of occurrence i n 1000 bootstrapped trees. The bar indicates a distance of 0.02 substitutions per site.

Beg et al., 2001). Two isolates from Pulau Seribu and Cirebon were identified as *Streptomyces bikiniensis*. *Streptomyces bikiniensis* produced the aminoglycosidic antibiotic (El-khawaga & Megahed, 2012).

Based on sampling location, 16 isolates of *Streptomyces* were obtained from Pulau Seribu, in which 15 of them were identified as different species. In Manokwari, it was found 13 isolates of *Streptomyces*, in which 8 of them showed different species. In Pelabuhan Ratu, 10 isolates of *Streptomyces* were obtained, in which 9 of them showed different species. In Garut 9, isolates of *Streptomyces* were obtained, all of them showed

different species. In Cirebon, 6 isolates of *Streptomyces* were obtained, also all of them showed different species. This suggests the extremely high genetic diversity of Indonesian marine *Streptomyces*. All of the isolates identified in this study were subsequently tested for antimicrobial activity. The molecular identification of all isolates up to the species level as well as their antimicrobial activities were presented in Table 1. The 16S rDNA-based phylogenetic tree of all isolates was presented in Figure 1.

Antimicrobial activity assays, as shown in Table 1, indicated that of 71 isolates tested, 23 isolates inhibited *B. subtilis* ATCC 66923, 14 isolates were active against *S. aureus* ATCC25923, 24 isolates were active against *C. albicans* BIOMCC 00122 and 26 isolates were active against *A. niger* BIOMCC 0013. None of the isolates showed inhibition to the growth of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. According to Kokare et al. (2004), during the screening of the novel secondary metabolites, *Streptomyces* isolates often show higher antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria. Eighty three percent of *Streptomyces* isolated from Sagamy Bay were found to have antifungal activities (Okami & Okazaki, 1972). Many marine microorganisms showed antifungal activity against *A. niger* (Kokare et al., 2004). Similarly, in this study, we found that the number of *Streptomyces* isolates able to inhibit *A. niger* are more than that showing the growth inhibition towards Gram-positif bacteria.

Figure 1. showed a neighbor-joining phylogenetic tree based on the analysis of 16S rRNA gene sequences. This suggests the phylogenetic relationship of isolates within related genera. Bootstrap values are expressed as percentages of 1000 replications. The percentage of replicate trees in which the associated taxa clustered together in boostrap are shown next to the branches. The phylogenetic tree showed that *Streptomyces* isolates originated from the same geographically region was not grouped into the same cluster. This suggests that a phylogenetic cluster may contain isolates of the same *Streptomyces* species, but from geographically different locations. For example, *Streptomyces* derived from Manokwari spread evenly in all clusters of the phylogenetic tree. A similar phenomenon was also observed for *Streptomyces* isolates originated from Pelabuhan Ratu, Garut, and Pulau Seribu. Thus genetic relationship between *Streptomyces* isolates did not correlate with the geographical location where the isolates obtained from. In contrast, some isolates of the same species came from two different sampling locations. For example, *Streptomyces albidoflavus* were found in two different locations, namely Manokwari and Pelabuhan Ratu, *Streptomyces cavourensis* were found in Garut and Cirebon.

4. Conclusion

This study revealed the extremely high diversity of marine *Streptomyces* in the Indonesian coastal waters. Of the seventy one isolates of *Streptomyces* tested for antimicrobial activities, it was found that 23 isolates were active against *B. subtilis* ATCC 66923, 14 isolates were active against *S. aureus* ATCC 25923, 24 isolates were active against *C. albicans* BIOMCC 00122 and 26 isolates were active against *A. niger* BIOMCC 0013. None of the isolates inhibited the growth of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Phylogenetic analysis showed that *Streptomyces* isolates originated from the same geographically region was not necessarily grouped into the same cluster. Likewise a phylogenetic cluster may contain isolates of the same *Streptomyces* species, but from geographically different locations.

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