BIODIVERSITAS
Volume 23, Number 3, March 2022
Pages: 1392-1398

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230325

Antimicrobial activity of *Streptomyces* spp. sponge-associated isolated from Samalona Island of South Sulawesi, Indonesia

HERLINA RANTE[1,♥], GEMINI ALAM[2], USMAR[3], SYARIFA ZAHRA[4], ARI KURNIAWATI[4], ALIMUDDIN ALI[5]

[1]Laboratory of Microbiology, Faculty of Pharmacy, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-588556, Fax.: +62-411-590663, ♥email: herlinarante@unhas.ac.id; h_rante@yahoo.co.id
[2]Phytochemical Laboratory, Faculty of Pharmacy, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[3]Pharmacology Laboratory, Faculty of Pharmacy, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[4]Faculty of Pharmacy, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[5]Laboratory of Microbiology Department of Biology, Universitas Negeri Makassar. Jl. Dg Tata Raya Parangtambung, Makassar 90223, South Sulawesi, Indonesia

Manuscript received: 28 July 2021. Revision accepted: 21 January 2022.

Abstract. *Rante H, Alam G, Usmar, Zahra S, Kurniawati A, Ali A. 2022. Antimicrobial activity of* Streptomyces *spp. sponge-associated isolated from Samalona Island of South Sulawesi, Indonesia. Biodiversitas 23: 1392-1398.* The resistance case of infectious diseases increases annually, while the necessity of antibiotics as a drug choice for therapy most important is needed. One of the efforts to overcome these problems is an investigation of new antibiotic sources, namely marine organisms. Actinomycetes are microbes well known as more antibiotic-producing than fungi and bacteria. This study aims to the identification of marine actinomycetes isolated from sponge samples in Samalona Island, South Sulawesi, Indonesia. The antimicrobial activity was evaluated against multi-drug resistant (MDR) *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Isolation was carried out using SCA (Starch Casein Agar) medium by the pour plate method. The selected isolates were designated as SML10h and SML10a. The isolates SML 10h and SML 10a were recovered from sponges *Haliclona* sp. and *Aplysina* sp., respectively. The production of antimicrobial substances was performed by fermentation of isolates in SNB (Starch Nitrate Broth) medium for 14 days. The antimicrobial activity was shown by the ethyl acetate extract with 2 mg/20 µL concentration against all test microbes. Phylogenetic analysis of the 16S rRNA gene sequences revealed that the similarity of SML 10a was most closely related to *Streptomyces geysiriensis* (98%).

Keyword: 16S rRNA gene, actinomycetes, antimicrobes, sponge,

INTRODUCTION

Indonesia is a well-known country as the largest archipelago in the world, located in the tropics with the highest marine organism biodiversity (mega biodiversity) (Rintelen et al. 2017). In recent years, the potential of marine biota as a source of the new bioactive compound has been widely studied, although not as much as on terrestrial biota. The long history of evolution in marine organisms causing ability to develop their adaptability through the formation of very high molecular diversity. Varying of compounds structure with various bioactivity was found from this biota such as antibacterial, antifungal, antiviral, anti-plasmodium and soon (Imhoff et al. 2011; Blunt et al. 2013). Among various marine biota, sponges are the organisms producing the most bioactive substance (Shreadah et al. 2008).

As reported, sponges are the largest source of bioactive compounds, producing most of the new metabolites than other marine organisms. Therefore, almost 30% of all-natural marine compounds are recovered from this organism (Brinkmann et al. 2017). Interestingly, these organisms harbor a wide variety of microorganisms which reach 60% of the total sponge biomass (Wilkinson 1980). In recent decades, the association between sponges and microorganisms has been most interesting research topic. This is due to the abundant production of chemically diverse secondary metabolites, which in nature serve as functional compounds required by marine sponge hosts. The production of bioactive natural substances by microorganisms is an important strategy to get various biologically active natural products in large quantities through selected microorganisms. Bioactive natural products from marine bacteria associated with sponges are the potential sources to be investigated to find new antibiotics in an effort to eradicate human microbial pathogens (Anand et al. 2012).

Natural products were found from various sources (including microorganisms, animals, and plants) could be used as drugs to overcome conventional medicine failure (Law et al. 2020; Sorokina and Steinbeck 2020). The ability of microorganisms to produce bioactive compounds on a large scale for overcoming the limitations of medicinal compounds by harvesting in large quantities of culture (Molinski et al. 2009). Marine microorganisms are the main sources for the discovery of bioactive compounds, an effort to provide new compounds both in terms of structure and bioactivity (Stien 2020). Among marine

microorganisms, actinomycetes are producers of bioactive compounds widely known different biological activities such as antitumor, antibiotic, immunosuppressive, antioxidant, antiviral, and enzyme inhibitory properties (Dharmaraj 2010).

Actinomycetes strains showed genetic potential to produce 10-20 secondary metabolites (Janardhan et al. 2014). Actinomycetes are highly considered antibiotic producers. Currently, approximately 70% of available antibiotics are isolated from actinomycetes (Almalki 2020). The purpose of this study was to investigate actinomycetes isolated from the sponge *Haliclona fascigera* recovered from Spermonde Island, South Sulawesi, and evaluate their antibacterial activity against multi-drug resistant (MDR) *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Sample collection

The sponge samples *Haliclona* sp. and *Aplysina* sp. were collected from Spermonde Archipelago, especially Samalona island South Sulawesi, Indonesia. The sponges were taken at a depth of 7-10 m below the sea level, with coordinates 05º.7'.20.28" (S) and 119º.20'.22.48" (E).

Isolation of actinomycetes

The sponge samples were washed with seawater, then the samples were cut 1 cm^2 aseptically. Samples were soaked in alcohol 70% for 5 minutes, then transferred in bottles containing sterile water and shaken gently. The samples were crushed in a sterile mortar until make pastel. The samples were heated at 70°C for 30 minutes, then diluted by decimal serial dilution.

A total of 1 mL of the final diluted sample was spread onto the SCA (Starch Casein Agar) media in a petri dish. The samples were incubated at room temperature for 21 days. The actinomycetes colony was streaked into a new SCA medium until obtained a purified colony. The purified colony was transferred to the ISP2 (International Streptomyces Project) slank medium for future investigation.

Antagonistic test of actinomycetes

The actinomycetes isolates were cultured in ISP2 agar media, then the agar block of cultures was cut by using a stainless steel cork borer. Test microorganisms were inoculated in a petri dish onto the NA and PDA for bacteria and fungi, respectively. The agar blocks were put on the surface of the media were culturing test microorganisms ~~m~~. The inhibition zone was observed surrounding the actinomycetes agar block after incubation at 37°C for 24 h was measured.

Fermentation and extraction

The active isolates were cultured in a 250 mL Erlenmeyer flask, which contained 100 mL of SNB (Starch Nitrate Broth) at room temperature for 3 days. After that, the seed cultures were transferred into 500 mL Erlenmeyer containing 100 mL SNB (pH 7.4). Fermentation was carried out at room temperature for 11 days in a shaken at 150 rpm. After the fermentation period was achieved, fermentation liquid was filtered to separate with biomass. The fermented liquid was extracted 2 times with ethyl acetate (1: 1 v/v) in a separating funnel. The ethyl acetate phase was evaporated to find extract then stored in a desiccator for use in subsequent tests.

Antibacterial activity

The antimicrobial activity of ethyl acetate extracts was evaluated using agar diffusion methods with a concentration of 2 mg/20 µL. A total of 20 µL of the test extract solution was dripped on 6 mm in diameter of the paper disk. The solvent was evaporated, then the paper disk was placed on the surface of the NA and PDA plate media after inoculating test bacteria and fungi, respectively. An inhibitory zone was observed around the paper disks, the diameter of the inhibition zone was measured.

Morphological and physiological characterization

Actinomycetes isolates were characterized morphologically and physiologically according to the ISP (International Streptomyces Project) (Shirling and Gottlieb 1966) procedure. Isolate Actinomycetes showed the highest antibacterial activity was characterized morphologically by using the culture slide method. The isolate was grown on SNA plate medium at room temperature for 7 days, then sterile slide glass was placed on the medium with a 45° slope around the microbial colony. After the strain mycelia grew on the surface of the object-glass, the spore chain was observed under a microscope at 400x magnification. Subsequently, the strain was characterized the physiological based on the utilization of Carbon (C) and Nitrogen (N) sources.

Molecular characterization of actinomycetes isolates using the 16S-rRNA gene

Preparation of DNA

Isolation of DNA was carried out by using Song et al. (2004) method. One milliliter of 7-day-old actinomycetes culture was pipetted into a micro sterile tube, centrifuged at 13,000 rpm for 5 minutes, and then discarded the supernatant. The pellets were washed with TE (Tris EDTA) 400 µL, centrifuged at 13,000 rpm for 5 minutes, re-suspended with 400 µL buffer SET (75 mM NaCl). The pellet was incubated on water bath 37°C for 1 hour, homogenized by inversion every 15 minutes, then added 50 µL SDS 10%, 20 µL protease and RNA-ase 50 µL, incubated at 65°C in the water bath for 2 hours. After incubation periods were achieved, pellets were added 167 µL NaCl 5 M. Approximately 400 µL of cold chloroform was added, then incubated at room temperature for 30 minutes, inverted every 10 minutes, then centrifuged at 13,000 rpm (10 minutes). The aqueous phase was transferred into a new tube, added isopropanol or 2-propanol 1x volume of the supernatant, was homogenized carefully, stored at -20°C (overnight), and then allowed until the room temperature achieve and centrifuged 13,000 rpm (5 minutes). The supernatant was removed, added with 100 µL of 70% ethanol and then homogenized, the ethanol

is removed and the pellet was dried. The pellets were dissolved in TE 25 µL. The purity of the DNA solution was checked by using a spectrophotometer at λ 260 and λ 280 nm, the amount of DNA measured at λ 260 nm.

Amplification of 16S rDNA sequences and phylogenetic tree analysis

16S rDNA gene sequenced by using the PCR method with DNA polymerase and primers 27f (5'AGAGTTTGAT CCTGGCTCAG-3') and 1492r (5'GGTTACCTTGTTA CGACTT-3'). The thermal conditions of the cycle as follows; denaturation of target DNA at 98°C for 3 minutes followed by 30 cycles at 94°C for 1 minute, primary annealing at 54°C for 1 minute, and primary extension at 72°C for 5 minutes and then cooled at 4°C. PCR amplification was detected using agarose electrophoresis gel and visualized by UV fluorescence after staining with ethidium bromide. The PCR products obtained were subsequently sequenced. The sequence was compared with genomes from the gene-bank database and the phylogenetic tree was constructed by using NCBI BLAST (www.ncbi.nih.gov/.) program.

RESULTS AND DISCUSSION

Location

The location of the research area in Spermonde Archipelago. It consists of a group of islands located in the Southwest of Sulawesi Island, Indonesia. There are approximately 130 islands that are included in the Spermonde Archipelago, one of which is Samalona Island as a sampling site. (Figure 1).

Actinomycetes isolate

In this study, the sponge samples were pretreated by heat-shock at 70°C for 30 minutes to kill the non-spore forming bacteria, whereas the nystatin was amended to the SCA medium to suppress mold growth. The actinomycetes were recovered from 2 sponge genera, namely *Haliclona* sp. and *Aplysina* sp. (Figure 2). The ability of isolate was grown on the isolation medium after about 2 to 3 weeks of incubation periods. As reported by Kamjam et al. (2017), the actinomycetes strain isolated from marine sediments achieved an incubation period of 3 weeks at room temperature, while Zhang et al. (2015) required an incubation period of 2-4 weeks at 28°C. Therefore, the supplemented of nystatin and heat sock pretreated are the most important procedure that aims to suppress the growth of unwanted bacteria and fungi (Ulanova and Goo 2015). The number of isolates was recovered by using Starch Nitrate agar with seawater shows that the medium most suitable for isolation of marine actinomycetes. This result was correlated with a study conducted by Rante et al. 2017, the Starch Nitrate, Starch-Casein, and Glycerol glycine media are the most suitable media to isolate actinomycetes bacteria. Based on the result, there are two selected actinomycetes recovered from *Haliclona* sp. and *Aplysina* sp. sponge, and the isolates were designated as SML10h and SML 10a, respectively.

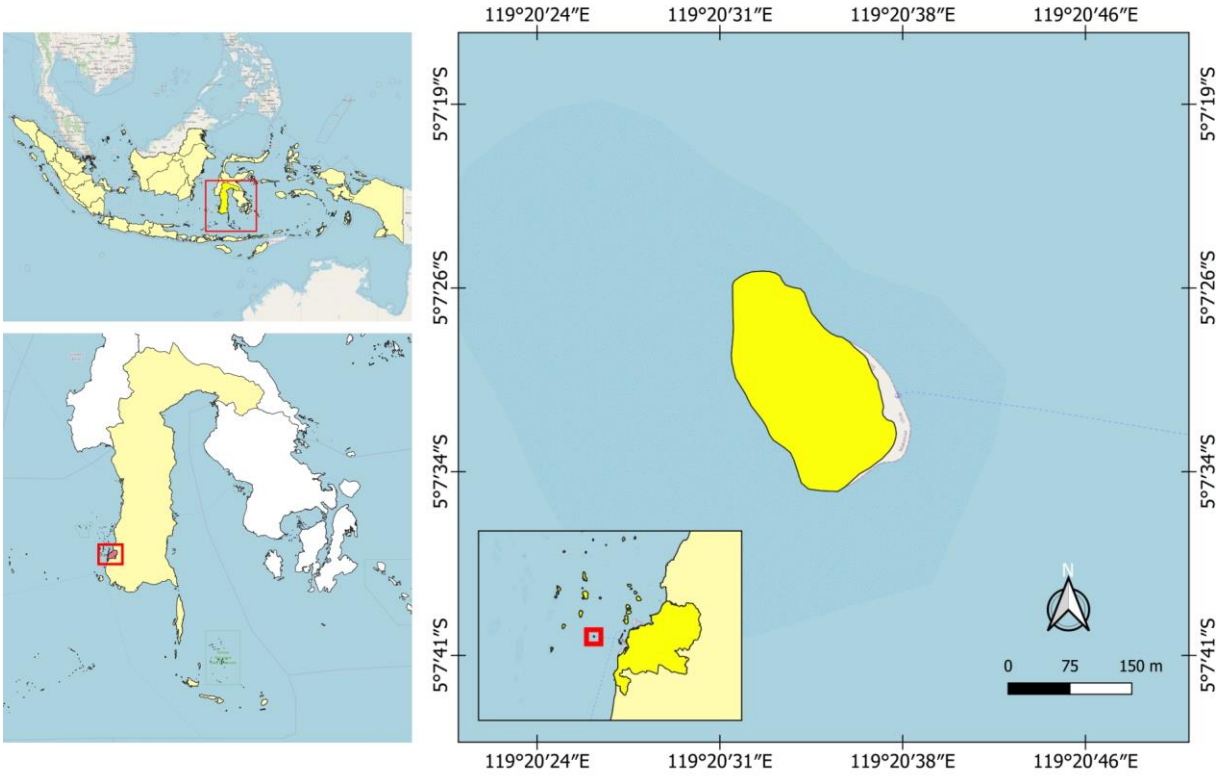

Figure 1. Samalona Island of Spermonde Archipelago, South Sulawesi Province, Indonesia (Haya 2017)

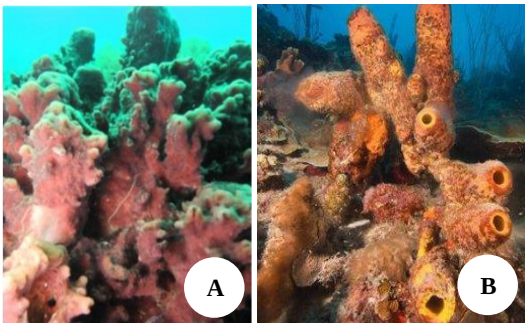

Figure 2. Sample sponge. A. *Haliclona* sp.; B. *Aplysina* sp.

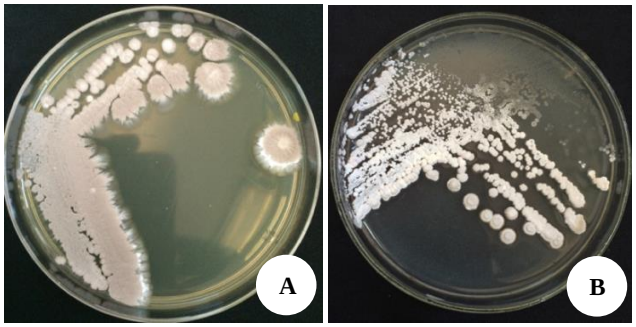

Figure 3. Morphological colony of Actinomycetes isolated from sponges of (A) *Haliclona* sp. and (B) *Aplysina* sp. was grown on SCA media after incubated for 7 days at room temperature

Table 1. Antagonistic test of actinomycetes isolates against the growth of test microbes

Actinomycetes isolates	Microbial test growth		
	***Staphylococcus aureus* MDR**	***Escherichia coli* MDR**	***Candida albicans***
SML 10h	+	+	+
SML 10a	+	+	+

Note: (+): Clear zone formed

Table 2. The antimicrobial activity of ethyl acetate extract of actinomycetes isolates against test microorganisms

Isolates	Microbial test growth (mm)		
	***Staphylococcus aureus* MDR**	***Escherichia coli* MDR**	***Candida albicans***
SML 10h	10.07	9.97	8.66
SML 10a	12.42	16.03	9.71

The antagonistic activity was showed actinomycetes inhibit the growth of test microorganisms. The antagonist test purpose is to find early data about the potential of the isolates before continuing to lab-scale production of metabolites by fermentation (Table 1).

Fermentation of actinomycetes isolates

Antibacterial compounds were produced by using SN broth media. The ability of isolates to produce metabolite was affected by the fermentation media components such as carbon, nitrogen and minerals. The carbon and nitrogen sources of SNB media from soluble starch and KNO3, respectively. The minerals are derived from magnesium, sodium, iron, potassium, which is a component of SNB media. The metabolic rate of carbon sources affects the formation of biomass and metabolites. Thus the use of carbon-nitrogen sources is one of the main factors to increase the secondary metabolites produce (Kim et al. 2021).

The antimicrobial activity of ethyl acetate extract was shown to inhibit all of the test microorganisms growth (Table 2). It is correlated with an antagonistic activity described previously, however, the ethanol extract does not inhibit all of the test microorganisms. It may be related to the polarity of active substances. The active substances of the extract are more non-polar than polar, so the ethyl acetate solvent is most suitable to dissolve the active substances, on the contrary with ethanol. In conclusion, the ethyl acetate extract could be grouped as a broad spectrum metabolite activity because it can inhibit both Gram-positive or Gram-negative and fungi.

Characterization of morphology and ornament of isolate spore chain producing antibacterial compounds

Morphological characteristics of isolates were observed of colonies grown on SNA (Starch Nitrate Agar) medium. The spore chain ornaments by using slide culture were observed under a microscopic at 100x magnification (Figure 4 and 5).

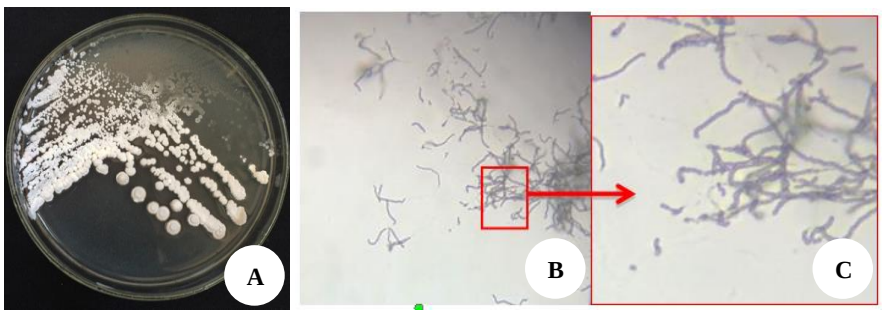

Figure 4. Morphological characteristics of SML 10a isolates. A. appearance of isolate colonies grown on SNA medium; B. Open spiral spore chain formation at 40x magnification; C. spore chain formation at 100x magnification

Although the isolates are similar of colony appearances, however the fundamental difference was shown in the formation of strike spore chain of SML 10h isolate, while SML 10a spore chains are open spiral.

The SML 10a isolate showed long and branched hyphae, and in certain hyphae showed a spiral shape, whereas SML 10h is in the form of a spiral biverticilus (BIV-S) which is a spiral shape, but the branching is rather dense than the main chain, which is characterized by its hairy, grayish-white color, and is wart-shaped and shows long and branched hyphae. The results of these observations indicate that SML 10a isolate and SML 10h isolate belongs to the genus *Streptomyces*.

According to Li et al. (2016), characteristics of the genus Streptomyces are produced distinctive aerial mycelium, which slowly forms spiral characteristics. Morphology of spore chains can be classified as straight, flexible, or spiral, while the surface of spores can be divided into smooth, warty, thorny, or hairy. Several methods to identify Streptomyces such as colonies type and mycelia spore chain. Streptomyces is common of dry and small colonies with a diameter of 1-10 mm. Furthermore, it can be identified by color grouping specific media. For example, the Oatmeal Agar (ISP-3) media, Streptomyces, produce a variety of different colors in both vegetative mycelium and aerial mycelium.

Table 3. The ability of selected actinomycetes isolates, S10a and S10h strains to utilize various carbon and nitrogen sources

Biochemical Test	Isolate codes	
	SML 10a	SML 10h
C Source Test		
D-Glucose (Control +)	++	++
Sucrose	++	++
Sorbitol	+	+
Mio-inositol	+	+
Dulsitol	+	++
D- Manitol	+	+
Starch	++	++
Dextrose	+	++
N Source Test		
ISP-2 (Control +)	++	++
L-valine	+	++
L-asparagine	+	+
L-Tyrosine	+	+
$(NH_4)_2SO_4$	+	+
Isoleucine	+	++
Urea	+	±
KNO_3	++	+
Glycine	++	++
Cysteine	+	-

Note: +: growth; -: not growth

Physiological and biochemical characterization

In addition to the morphological characteristics, the physiological and biochemical characteristics are considered most important for characterizing microbial isolate. Biochemical and physiological analyses include selected isolates' ability to utilize various carbon and nitrogen sources available to support their growth (Table 2).

Molecular characterization of actinobacteria isolates using the 16S-rRNA gene

16S rRNA molecular analysis of selected isolates was carried out by gene sequencing and phylogenetic tree analysis. Blast-n purpose to find out the relationship between the reference strains. The results of the 16S rRNA gene analysis with reference strains were constructed phylogenetic trees (Figure 7). BLAST results show the isolated SML 10a highest similarity with *Streptomyces geysiriensis* (98%) illustrated in phylogenetic tree reconstruction.

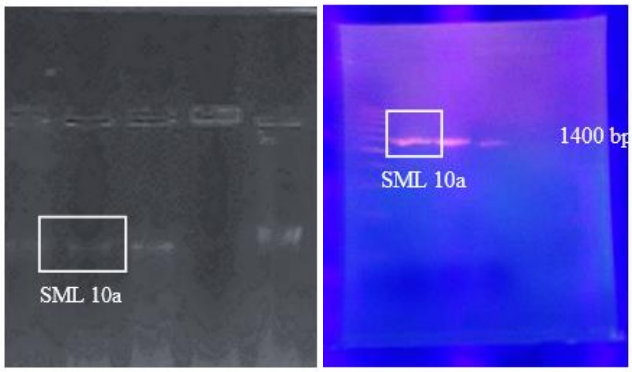

Figure 6. Agarose gel electrophoresis result of the 16S rRNA PCR of Actinomycetes SML 10a

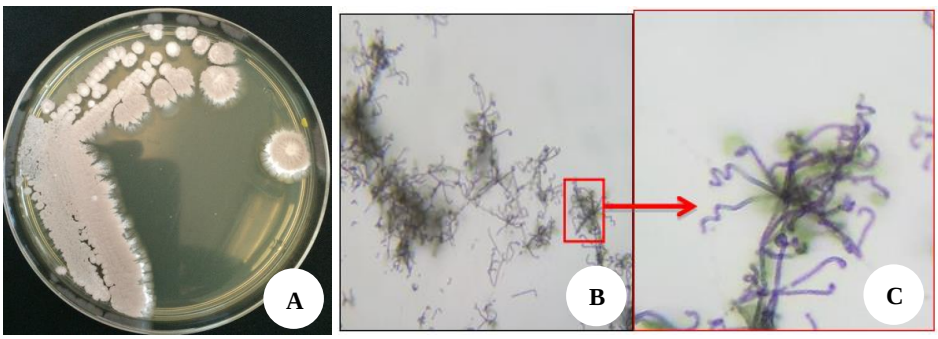

Figure 5. Morphological characteristics of SML 10h isolate. A. Appearance of the colony grown on SNA medium; B. Biverticillus spiral spore chain formation (BIV-S) at 40x magnification; C. Spore chain formation at 100x magnification

Table 4. Data from the BLAST Alignment program results

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces geysiriensis strain NRRL B-12102 16S ribosomal RNA gene, partial sequence	2370	2370	98%	0.0	98%	NR_043818.1
Streptomyces geysiriensis strain NBRC 15413 16S ribosomal RNA gene, partial sequence	2370	2370	98%	0.0	98%	NR_112459.1
Streptomyces ghanaensis strain CSSP718 16S ribosomal RNA gene, partial sequence	2370	2370	98%	0.0	98%	NR_043366.1
Streptomyces rochei strain NRRL B-1559 16S ribosomal RNA gene, partial sequence	2364	2364	98%	0.0	98%	NR_116078.1
Streptomyces enissocaesilis strain NRRL B-16365 16S ribosomal RNA gene, partial sequence	2364	2364	98%	0.0	98%	NR_115668.1
Streptomyces vinaceusdrappus strain NBRC 13099 16S ribosomal RNA gene, partial sequence	2364	2364	98%	0.0	98%	NR_112368.1
Streptomyces plicatus strain NBRC 13071 16S ribosomal RNA gene, partial sequence	2364	2364	98%	0.0	98%	NR_112357.1
Streptomyces rochei strain NBRC 12908 16S ribosomal RNA gene, partial sequence	2364	2364	98%	0.0	98%	NR_041091.1
Streptomyces mutabilis strain NRRL ISP-5169 16S ribosomal RNA gene, partial sequence	2344	2344	98%	0.0	97%	NR_044139.1
Streptomyces mutabilis strain NBRC 12800 16S ribosomal RNA gene, partial sequence	2344	2344	98%	0.0	97%	NR_112281.1
Streptomyces diakartensis strain NBRC 15409 16S ribosomal RNA gene, partial sequence	2337	2337	98%	0.0	97%	NR_041178.1
Streptomyces tuirus strain NBRC 15617 16S ribosomal RNA gene, partial sequence	2337	2337	98%	0.0	97%	NR_041190.1
Streptomyces anandii strain JCM 4720 16S ribosomal RNA gene, partial sequence	2335	2335	98%	0.0	97%	NR_041135.1
Streptomyces tuirus strain ICSSB 1017 16S ribosomal RNA gene, partial sequence	2333	2333	98%	0.0	97%	NR_114666.1
Streptomyces vinaceusdrappus strain NRRL 2363 16S ribosomal RNA gene, partial sequence	2331	2331	98%	0.0	97%	NR_043383.1
Streptomyces naganishii strain NRRL B-1816 16S ribosomal RNA gene, partial sequence	2329	2329	98%	0.0	97%	NR_043831.1

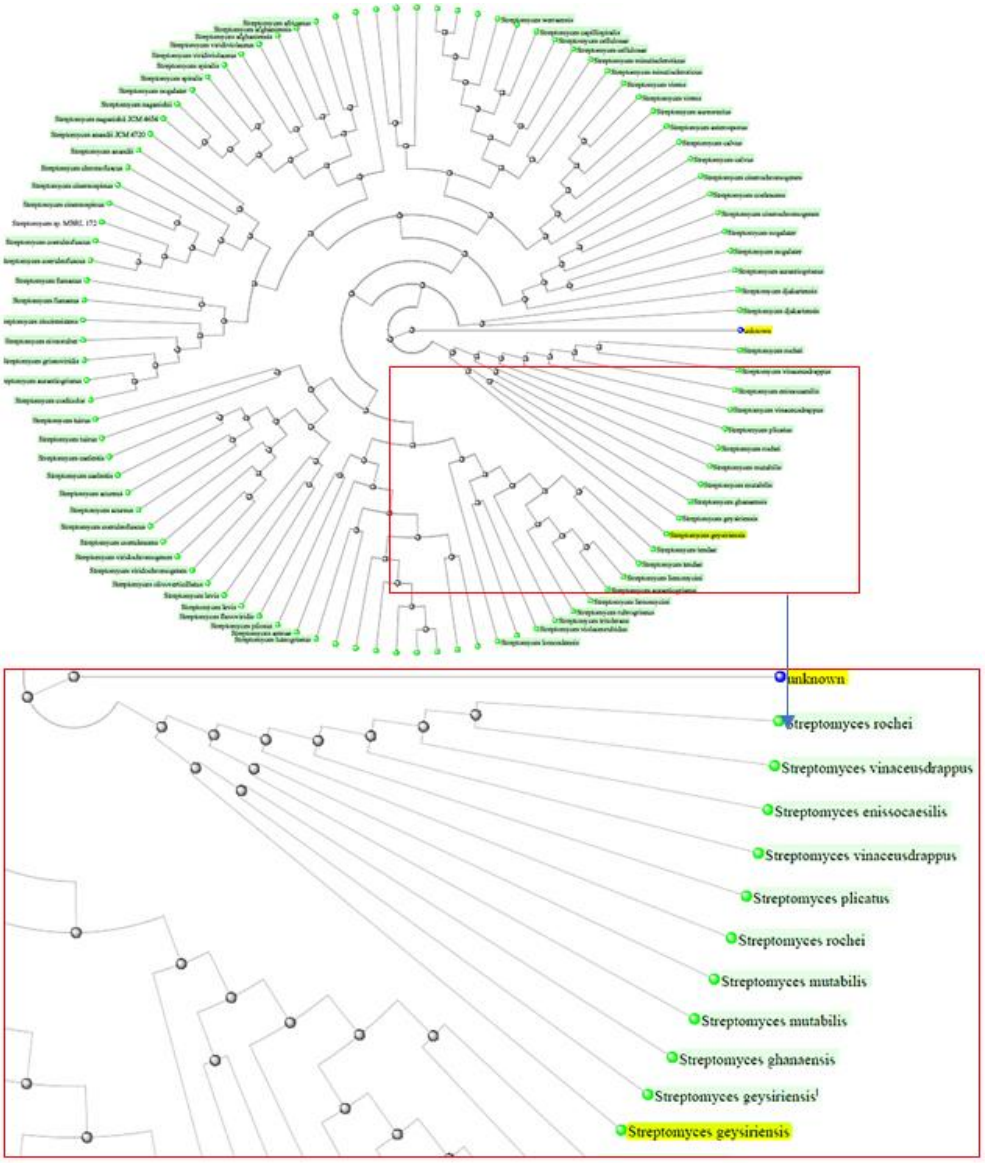

Figure 7. Analysis of the phylogenetic tree of Actinomycetes isolates of SML 10a based on the 16S-rRNA gene

In conclusion, the SML 10a and SML 10h actinomycetes were isolated from a sponge at Samalona Island, South Sulawesi, Indonesia are inhibit all the test microorganisms. The ethyl acetate extract of SML 10a inhibits *S. aureus*, *E. coli*, and *C. albican*s at a concentration 2 mg/20µL. The phylogenetic tree of the isolate is most closely related to *Streptomyces geysiriensis* (similarity 98 %).

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Research, Technology and Higher Education, Indonesia in 2019 through Higher Education Foundation Basic Research Grant Scheme. The authors declare that they have no conflicts of interest.

REFERENCES

Almalki MA. 2020. Isolation and characterization of polyketide drug molecule from *Streptomyces* species with antimicrobial activity against clinical pathogens. J Infect Public Health 13 (1): 125-130. DOI: 10.1016/j.jiph.2019.07.002.

Anand P, Shanthini CF, Chellamaram C.2012. Screening for herbicidal and growth promotor activities in marine bacteria. Intl J Pharm Bio Sci 3 (2): 659-668.

Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. 2013. Marine natural products. Nat Prod Rep 30: 237-323. DOI: 10.1039/c2np20112g.

Brinkmann CM, Marker A, Kurtboke Di. 2017. An overview on marine sponge-symbiontic bacteria as un exhausted sources for natural product discovery. Diversity 9 (4): 40. DOI: 10.3390/d9040040.

Dharmaraj S. 2010. Marine *Streptomyces* as a novel source of bioactive substances. World J Microbiol Biotechnol 26 (12): 2123-2139. DOI: 10.1007/s11274-010-0415-6.

Haya LOMY, Fujii M. 2017. Mapping the change of coral reefs using remote sensing and in situ measurements; A case study in Pangkep Regency, Spermonde Archipelago, Indonesia. J Oceanogr 73 (5): 623-645. DOI: 10.1007/s10872-017-0422-4.

Imhoff JF, Labes A, Wiese J. 2011. Bio-mining the microbial treasures of the ocean: New natural product. Biotechnol Adv 29 (5): 468-482. DOI: 10.1016/j.biotechadv.2011.03.001.

Janardhan A, Kumar AP, Viswanath B, Saigopal DVR, Narasimha G. 2014. Production of bioactive compounds by actinomycetes and their antioxidant properties. Biotechnol Reas Intl 2014: 217030. DOI: 10.1155/2014/217030.

Kamjam M, Sivalingam P, Deng Z, Hong K. 2017. Deep sea Actinomycetes and their secondary metabolites. Front Microbiol 8: 760. DOI: 10.3389/fmicb.2017.00760.

Kim JH, Lee N, Hwang S, Kim W, Lee Y, Cho S, Palsson BO, Cho BK. 2021. Discovery of novel secondary metabolites encoded in actinomycete genomes through coculture. J Ind Microbiol Biotechnol xx 48: kuaa001. DOI: 10.1093/jimb/kuaa001.

Law JWF, Law LNS, Letchumanan V, Tan LTH, Wong SH, Chan KG, Ab Mutalib NS, Lee LH. 2020. Anticancer drug discovery from microbial sources: The unique mangrove *Streptomycetes*. Molecules 25 (22): 5365. DOI: 10.3390/molecules25225365.

Molinski TF, Dalisay DS, Lievens SL, Saludes JP. 2009. Drug development from marine natural products. Nat Rev Drug Discov 8 (1): 69-85. DOI: 10.1038/nrd2487.

Rante H, Yulianty R, Usmar, Djide N, Subehan, Burhamzah R, Prasad MB. 2017. Actinomycetes of *Orthosipon stamineus* rhizosphere as producer of antibacterial compound against multidrug resistant bacteria. IOP Conf Ser: Mater Sci Eng 259: 012003. DOI: 10.1088/1757-899X/259/1/012003.

Rintelen KV, Arida E, Hauser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Res Ideas Outcomes 3: e20860. DOI: 10.3897/rio.3.e20860.

Shirling EB, Gottlieb D. 1966, Methods for characterization of *Streptomyces* species. Intl J Syst Bacteriol 16: 313-340. DOI: 10.1099/00207713-16-3-313.

Shreadah MA, Masoud MS, Said TO, El Zokm GM. 2008. Aplication of IR, X-Ray, TGA and DTA to determine the mineral composition of the sediments and study of reaction kinetics along the Egyptian Red Sea Coasts. Egypt J Aquat Res 34 (2): 83-95.

Sorokina M, Steinbeck C. 2020. Review on natural products databases: Where to find data in 2020. Aust J Chem 12 (1): 20. DOI: 10.1186/s13321-020-00424-9.

Stien D. 2020.Marine microbial diversity as a source of bioactive natural products. Mar Drugs 18 (4): 215. DOI: 10.3390/md18040215.

Li Q, Chen X, Jiang Y, Jiang C. 2016. Morphological identification of Actinobacteria. In: Dhanasekaran D, Jiang Y (eds). Actinobacteria-Basics and Biotechnological Applications. IntechOpen, London.

Ulanova D, Goo K. S. 2015. Diversity of actinomycetes isolated from subseafloor sediments after prolonged low-temperature storage. Folia Microbiol 60: 211-216. DOI: 10.1007/s12223-014-0361-z.

Wilkinson CR. 1980. Cyanobacteria symbiotic in marine sponges. In: Schwemmler W, Schenck HEA (eds). Endocytobiology, Endosymbiosis and Cell Biology. De Gruyter, Berlin.

Zhang G, Zhang Y, Yin X, Wang S. 2015. *Nesterenkonia alkaliphila* sp. nov., an alkaliphilic, halotolerant actinobacteria isolated from the western Pacific Ocean. Intl J Syst Evol Microbiol 65: 516-521. DOI: 10.1099/ijs.0.065623-0.