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POTENCY OF ACTINOMYCETES FROM DEEPSEA SEDIMENT OF MAKASSAR STRAIT FOR PRODUCING ANTIMICROBIAL SUBSTANCES

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Abstract

A study on isolation of actinomycetes from sediments of Makassar Strait have been conducted with regard to research project called Widya Nusantara Exploration (EWIN) in May-June 2013 and November 2014. The objectives of this research were to screen antimicrobial activity of 36 actinomycetes from sediments of Makassar Strait, characterized the potential isolates, and determined the metabolites produced by the selected isolate. The antimicrobial screening was conducted using agar diffusion method, while characterization of the best five of actinomycetes were using APIZYM Kit, Scanning Electron Microscope, and FTIR. Five isolates retrieved from this research had ability to inhibit the growth of four microbial testing: *Escherichia coli*, *Bacillus subtilis*, *Staphyllococcus aureus* and *Candida albicans*. The highest capability was shown by the MACMK-43 isolate that had 16S rRNA gene sequence similarity of 97.85% to *Streptomyces violacens*. The result shows that, the active fraction contained of 4-amino-5-cyano-6-(4'methoxyphenyl)-1-methyl-2.3-dihydropyrrolo [2,3-B] pyridine, which is commercially used for bactericide and antihistamine.

Keywords: antimicrobes, actinomycetes, *Streptomyces violascens,* deepsea sediment

1. Introduction

Actinomycetes are the group of high G + C content gram positive bacteria, in which have an ability to produce diverse bioactive compounds, such as antibiotics, antifungals, antiparasitic, and anticancer drugs (Cragg, Kingston, & Newman, 2011; Helaly, Pesic, Fiedler, & Sussmuth, 2011; Gao et al., 2012; Lu et al., 2012). Up to now, 45% of the bioactive compounds produced by microbes were produced by actinomycetes, so actinomycetes are still major natural antimicrobial producer (Berdy, 2005). Manivasagan, Jayachandran, Kannan & Se-Kwon, (2013) reported that among 23.000 antimicrobial produced by microbes, more than 10.000 isolated from actinomycetes, especially Genera *Streptomyces.*

The decline in the discovery of new substances obtained from terrestrial actinomycetes has led scientists and researchers to focus on exploring actinomycetes from extreme environments. Marine is one of the environments that being studied as a source of actinomycetes isolation. The marine environment attracts the attention of researchers as a source of microbial isolation, and it has been reported that marine microbes including actinomycetes are useful for screening new secondary metabolites (Lam, 2006; Blunt et al., 2007; Khan et al., 2011).

Several studies have shown that actinomycetes isolated from the marine environment are metabolically active and have adapted to marine life (Bull, Stach, Ward, & Goodfellow, 2005; Lam, 2006; Valli et al., 2012). Marine actinomycetes are widely distributed

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in a variety of marine habitats from marine sand (Hong, Lee, Yim, Chun, & Lee, 2008), mangrove sediments (Hong et al., 2009; Hong, 2013; Azman, Othman, Velu, Cha, & Lee, 2015; Hamada et al., 2015a; Hamada et al., 2015b), seawater (Zhang, Xi, Ruan, & Huang, 2012), coastal sediments (Yu et al., 2015), and deep sea sediments (Pathom-aree et al., 2006; Bredholt et al., 2007 ; Luo et al., 2011; Zhang, Zhang, Yin, & Wang, 2015; Chen et al., 2016). The increasing number of studies on marine actinomycetes indicates that marine environment including the deep ocean, is a significant source for finding and discovering both the marine actinomycetes diversity and its secondary metabolites product. (Skropeta & Wei, 2014; Xu, Ye, Han, Deng, & Hong, 2014). In the last few years, several reports on the discovery of antimicrobial compounds of actinomycetes isolated from some deepsea sediments are shown in Table 1. These potential strains were isolated from marine sediments in deepsea of South China Sea and Indian Ocean. This phenomenon was confirmed by Kamjam Sivalingam, Deng, and Hong(2017), that the deep ocean is a hot spot for hunting the source of marine origin actinomycetes and its new secondary metabolites, due to its uniqueness and extreme environment.

A study on exploring antimicrobial producing actinomycetes obtained from the sediments with depth level between 150-3.366 meters of Makassar Strait have been done. This research aimed to screen antimicrobial activity of 36 actinomycetes from sediments of Makassar Strait, characterized the potential isolates, and characterized their metabolites product. The results of this study hopefully will enrich the information about potential actinomycetes from the deepsea waters of Indonesian territory.

2. Material and Methods

2.1. Microbial Strains

Thirty-six marine actinomycetes strains used in this study were come from collections of Marine Microbiology Laboratory of the Oceanography Research Center LIPI, Ancol and Indonesian Culture Collections (InaCC) Biology Research Center LIPI. The medium used for isolation were Actinomycetes Isolation Agar (AIA, Hymedia, India) and NBRC 802 media (Hamada, Shibata, Tamura, & Suzuki, 2013). All isolation media were added with nalidixic acid and cycloheximide to hamper bacterial growth (Hayakawa, Momose, Yamazaki, & Nonomura, 1996). Molecular identification of the isolates were conducted using analysis of 16S rRNA gene sequencing. The bacterial testing used were *Escherichia coli*, *Bacillus subtilis,*

Table 1. Actinomycetes isolated from deepsea sediments and its antimicrobial compounds

Antimicrobes	Name of Isolate	Source (deepsea level in m)	Reference
Pseudono-cardians A-C	*Pseudonocardia* sp	South China Sea (3,258 m)	Li et al., 2011
Indole alkaloid	*Serinicoccus profundi* sp. nov.	Indian Ocean (5,368 m)	Yang et al., 2013
Lobophorins H dan I	*Streptomyces* sp. 12A35	South China Sea (2,134 m)	Pan et al., 2013
Champacyclin	*Streptomyces* strain C42	Baltic Sea (241 m)	Pesic et al., 2013
Abyssomycins J-L	*Verrucosispora* sp.	South China Sea (2,733 m)	Wang et al., 2013
D. sotamides B-D	*Streptomyces scopuliridis*	South China Sea (3,536 m)	Song et al., 2014
Bafilomycins B1 & C1	*Streptomyces cavourensis* NA4	South China Sea (1.464 m)	Pan et al., 2015
Dehydroxy-aquayamycins	*Streptomyces* sp. SCSIO 11594	South China Sea (2.403 m)	Song et al., 2015

and *Staphylococcus aureus,* while fungal testing used was *Candida albicans.*

2.2. Fermentation and Extraction of Active Substance

Fermentation was carried out using medium of Starch Yeast Pepton Broth (SYP Broth), which contained of a 10 gr soluble starch, 4 gr yeast extract, 2 gr pepton, and aquadest up to 1000 mL of solution. Medium was sterilized in an autoclave at 121°C, 2 atm pressure, for 15 minutes, then settled at room temperature and ready to be used. One pure isolate was inoculated into the pre-culture medium containing 5 ml of SYP Broth medium, then incubated at 30°C for 2 days (48 hours) in the incubator shaker at 100 rpm. Thereafter, the entire pre-cultured medium was transferred into a fermentation medium containing 100 mL of SYP Broth and incubated at 30°C in a shaker incubator at 100 rpm for 10 days (240 hours). The cultures were then extracted by adding approximately 1:1 = media:ethyl acetate. The flasks were shaken at 30 °C and 100 rpm for 12 hours, then ethyl acetate fractions were transferred into new flasks. Extracts were evaporated using vacuum evaporator at temperature of 30 °C up to 10 ml. Raw material was then transferred into a vial, evaporated at room temperature to dry. 1 ml of methanol was added into each vial, and then dissolved and homogenized using vortex. The residual metabolite which was not soluble in methanol, was dissolved by 1 ml of DCM (Dichloromethane), and then homogenized using vortex. The extracts were stored at 4°C and ready to be screened.

2.3. Antimicrobial Screening

Antimicrobial screening was measured by agar diffusion method (Kanoh, Adachi, Katsuta, & Shizuri, 2008). The *E. coli, S. aureus,* and *B. subtilis* microbial testing used for screening were grown on Nutrient Agar (NA) and Nutrient Broth (NB) medium, while *C. albicans* was grown on Potato Dextrose agar (PDA) medium or Potato Dextrose Broth (PDB) medium. Microbial testing were incubated at 37 °C for 1 x 24 hours for *E. coli, S. aureus,* and *B. subtilis*, whereas for *C. albicans* for 2 x 24 hours. Petri dishes containing NA medium were smeared with each microbial testing of *E. coli, S. aureus,* and *B. subtilis*, while PDA medium was spread with *C. albicans*. Those pathogenic microbials were spreaded aseptically on surface of medium by using sterile cotton bud. A total of 15 mL of extract was dripped onto a 6 mm thick diameter paper disc, then dried in an aseptic laminar airflow. Each of the paper discs was then placed on agar medium that has been smeared with microbial testing. Petri dishes containing each pathogenic microbial were used as positive control. Paper discs containing of methanol and DCM solvent that put in the petri dishes containing with pathogenic microbial were used as negative control. The petri dishes were incubated at 37 °C for 2 x 24 hours, and observed for every 24 hours. Each emerging clear zone was measured and recorded as an inhibition zone.

2.4. Semi Quantitative Enzymatic Characterization

The enzymatic reaction test was performed using APIZYM strip (Biomereux). The system consists of a strip with 20 microwells, which its base contains of the enzymatic substrate and its buffer. The enzymatic tests are inoculated with a dense suspension of organisms, which is used to rehydrate the enzymatic substrates. The metabolic and products produced during the incubation period are detected through coloured reactions revealed by the addition of reagents.

About 5 ml of distilled aquadest was poured in an incubation box (a closed mica box) to create a moist atmosphere. The APIZYM kit strip was then placed in the incubation box. The inoculation of actinomycetes culture into the strip was performed using a sterile micropipette. A total of 65 µl of liquid actinomycetes cultures were introduced into each well. After inoculation, the incubation box was closed and incubated for 4-4.5 hours at 37 °C. After incubation, one drop of ZYM A reagent and one drop of ZYM B reagent were added to each well. The strips were stand about 5 minutes, then the colour formed and compared with the colour chart provided by manufacture (Humble, King, & Phillips, 1977).

2.5. Fractionation, Purification and Determination of Active Compounds

The fractionation and partial purification of the active compound was carried out by silica gel chromatography column using solvent hexane : ethyl acetate = 1 : 1, while the characterization of the active fraction was performed by using Gas Chromatography Mass Spectrophotometer (GCMS) (7890B GC, Agilent Technologies USA) with autosampler and Chemstation Data System. The peaks produced by this measurement was compared to library database in the tool.

Table 2. Results of antimicrobial assay of 36 strains of actinomycetes from deepsea sediment of Makassar Strait against *E. coli, B. subtilis, S. aureus,* and *C. albicans*

No	Strains code	Top Hit Taxon	Top Hit Strain	Identity (%)	Diameter (mm)			
					E. coli	*S. aureus*	*B. subtilis*	*C. albicans*
1	MACMK-08	*Micromonospora chalcea*	DSM43026^T	99.71	-	-	-	8
2	MACMK-09	*Gordonia didemni*	B204^T	98.52	-	-	-	7.5
3	**MACMK-14***	***Micrococcus yunnanensis***	**YIM 65004^T**	**99.78**	**26.33**	**1.2**	**0.78**	**26.6**
4	MACMK-19	*Micromonospora tulbaghiae*	DSM45142^T	99.18	-	-	-	8
5	MACMK-20	*Nocardiopsis alba*	DSM43377^T	99.93	-	-	-	-
6	MACMK-25	*Micromonospora tulbaghiae*	DSM45142^T	98.52	9.7	-	-	11.1
7	MACMK-32	*Micromonospora auratinigra*	DSM44815^T	98.83	-	-	-	11
8	**MACMK-37***	***Micromonospora chalcea***	**DSM 43026^T**	**99.64**	**19.9**	**1**	**0.7**	**22.3**
9	**MACMK-43***	***Streptomyces violascens***	**ISP 5183^T**	**97.58**	**21.13**	**1.2**	**0.7**	**28.95**
10	MACMK-52	*Micromonospora tulbaghiae*	DSM45142^T	99.27	1.19	-	-	
11	MACMK-53	*Micromonospora tulbaghiae*	DSM45142^T	99.35	0.88	-	-	10.7
12	MACMK-54	*Micromonospora tulbaghiae*	DSM45142^T	94.73	1.7	-	-	7.3
13	MACMK-55P	*Micromonospora tulbaghiae*	DSM45142^T	99.42	8.2	-	-	-
14	MACMK-55C	*Micromonospora chalcea*	DSM43026^T	99.64	-	-	-	-
15	MACMK-56	*Streptomyces diastaticus* subsp. *Diastaticus*	NBRC 3714^T	98.92	-	-	-	10.2
16	MACMK-57	*Arthrobacter subterraneus*	CH7^T	99.77	10.8	-	-	8.9
17	MACMK-58	*Streptomyces diastaticus* subsp. *Ardesiacus*	NRRL B-1773^T	99.64	-	-	-	8.2
18	MACMK-60	*Verrucosispora gifhornensis*	DSM44337^T	99.18	18.6	-	-	-
19	MACMK-61	*Micromonospora wenchangensis*	CCTCC AA 2012002^T	100	-	-	-	1
20	MACMK-62	*Micromonospora tulbaghiae*	DSM45142^T	99.48	-	-	-	-
21	MACMK-63	*Micromonospora tulbaghiae*	DSM45142^T	100	-	-	-	8
22	MACMK-64	*Micromonospora maritime*	D10-9-5^T	99.85	-	-	-	7.9
23	MACMK-65	*Verrucosispora fiedleri*	MG-37^T	99.56	-	-	-	10.3
24	MACMK-66	*Micromonospora chalcea*	DSM43026^T	99.71	-	-	-	-
25	MACMK-67	*Micromonospora chalcea*	DSM43026^T	99.71	-	-	-	7
26	MACMK-68	*Micromonospora tulbaghiae*	DSM45142^T	99.35	-	-	-	8.6
27	MACMK-69	*Micromonospora tulbaghiae*	DSM45142^T	99.11	-	-	-	9.6
28	MACMK-70	*Luteipulveratus halotolerans*	C296001^T	99.93	10.9	-	-	9.4
29	MACMK-71	*Verrucosispora gifhornensis*	DSM44337^T	99.71	9.3	-	-	7.5
30	**MACMK-72***	***Verrucosispora gifhornensis***	**DSM 44337^T**	**99.85**	**21.8**	**1.05**	**0.8**	**28.9**
31	MACMK-73	*Micromonospora tulbaghiae*	DSM45142^T	99.49	-	-	-	0.65
32	MACMK-74	*Verrucosispora gifhornensis*	DSM44337^T	99.86	-	-	-	7.7
33	MACMK-75	*Luteipulveratus halotolerans*	C296001^T	100	-	-	-	-
34	MACMK-77	*Micromonospora terminaliae*	TMS7^T	99.26	-	-	-	9.6
35	**MACMK-80***	***Kytococcus sedentarius***	**DSM 20547^T**	**99.78**	**17.9**	**0.95**	**0.7**	**21.9**
36	MACMK-81	*Micromonospora maritime*	D10-9-5^T	100	-	-	-	-

*Bold are the best five of actinomycetes strains; ^{T} are strain's type of top hits strain

2.6. Scanning Electron Microscopy

Morphological performance of strain *Streptomyces violascens* MACMK-43, obtained from deep floor sediments of Makassar Strait was observed by Scanning Electron Microscopy (JSM 5310 LV, JEOL Japan).

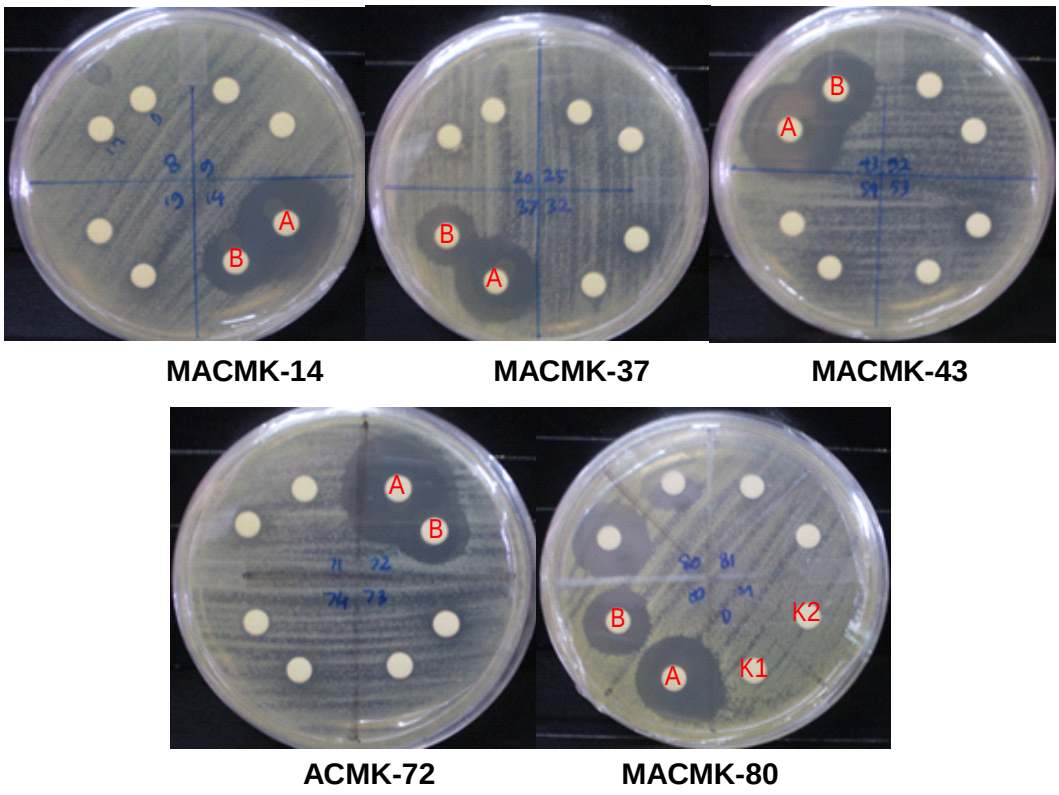


Figure 1. Clear zone showed by isolate MAMCK-14, MACMK-37, MACMK-43, MACMK-72 dan MACMK-80 against pathogenic microbial testing *C. Albicans* A: raw extract in methanol solvent, B: raw extract in DCM solvent, K1 : positive control 1 (solvent methanol), K2 : positive control 2 (DCM solvent).

3. Results and Discussion

3.1. Capability of Actinomycetes on Producing Antimicrobial Compounds

Thirty-six isolates of actinomycetes, which have been isolated from the Makassar Strait seafloor sediments with depths on between 150 and 3,366 meters, were divided into 9 genera including *Micromonospora, Verrucosispora, Streptomyces, Luteipulveratus, Nocardiopsis, Micrococcus, Gordonia, Kytococcus,* and *Arthrobacter* (Hatmanti, Lisdiyanti, Widada, & Wahyuono, 2018). The 36 isolates have been tested for inhibition of 3 pathogenic bacterial testing and 1 pathogenic fungus testing, i.e., *E. coli, S. aureus, B. subtilis* and *C. albicans*. The results are shown in Table 2. From 36 strains, 14 strains inhibited the growth of *E. coli*, 5 strains inhibited *S. aureus* and *B. subtilis*, and 27 strains inhibited *C. albicans*. Based on Table 2, five best isolates were selected which were capable of inhibiting all four types of pathogens and had greatly inhibitory power by showing larger clear zones than others. The strains were MACMK-14 (*Micrococcus yunnanensis* 99.78%), MACMK-37 (*Micromonospora chalcea*, 99.64%), MACMK-43 (*Streptomyces violascens*, 97.58%), MACMK-72 (*Verrucosispora gifhornensis*, 99.85%), and MACMK-80 (*Kytococcus sedentarius*, 99.78%). Figure 1 shows clear zone produced by five leading strains inhibiting *C. albicans*, compared to others. The clear zone indicates that actinomycetes strains retrieved from marine sediments of Makassar Strait are able to inhibit the growth of *C. albicans*. *Streptomyces violascens* MACMK-43 strain was selected for further analysis on producing antimicrobial compound.

3.2. Characterization of the Best Five Actinomycetes Producing Antimicrobial Compounds: The Enzymatic Activity

The enzymatic activity of the five selected strains was tested using APIZYM Kit (Biomereux). The photographs of the test results are shown in Figure 2 and the test readings are listed in Table 3. Semi quantitative readings show that there are two unique isolates, i.e.: MACMK-43 strain which has stronger and more diverse enzymatic activity against some

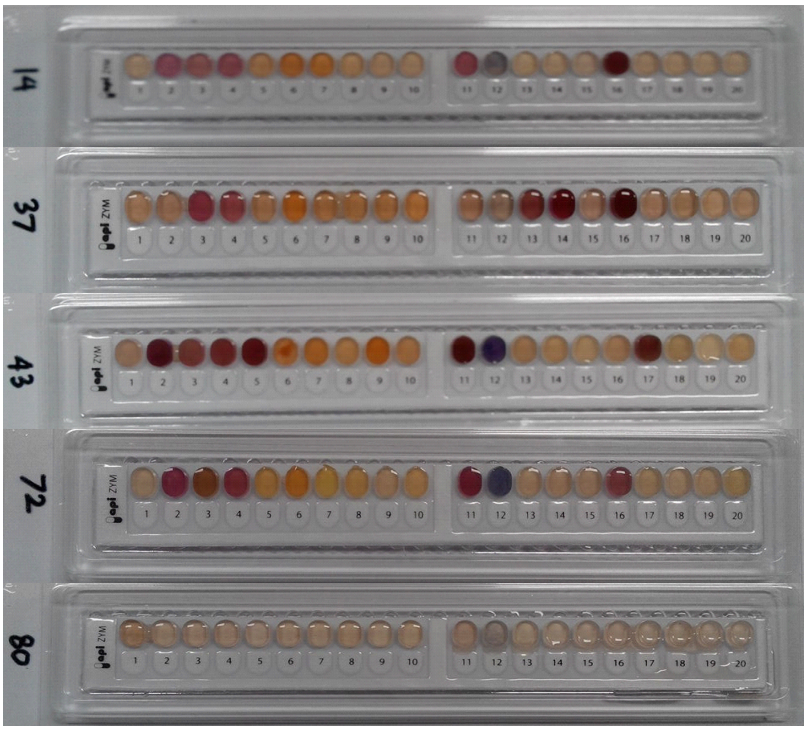


Figure 2. The enzymatic test of 5 preeminent isolates using APIZYM strip (Biomereux) i.e.: MACMK-14, MACMK-37, MACMK-43, MACMK-72 and MACMK-80.

Table 3. Results of enzymatic reaction of the best 5 isolates using APIZYM Kit, compared to colour chart provided by manufacture (Biomereux)

No	Enzyme	Quantity of hydrolysed substrate				
		MACMK-14	MACMK-37	MACMK-43	MACMK-72	MACMK-80
1	Control	0	0	0	0	0
2	Alkaline phosphatase	3	1	5	5	1
3	Esterase (C4)	3	5	3	5	1
4	Esterase Lipase (C8)	3	3	5	5	1
5	Lipase (C14)	1	1	5	1	0
6	Leucine arylamidase	5	3	5	3	0
7	Valine arylamidase	3	1	5	1	0
8	Cystine arylamidase	0	1	1	1	0
9	Trypsin	0	1	5	1	0
10	α-chymotrypsin	0	1	1	1	0
11	Acid phosphatase	3	1	5	5	1
12	Naphthol-AS-BI-phosphohydrolase	3	1	5	5	1
13	α-galactosidase	0	5	0	0	0
14	β-galactosidase	0	5	0	0	0
15	β-glucuronidase	0	0	0	0	0
16	α-glucosidase	5	5	0	5	1
17	β-glucosidase	0	0	5	0	0
18	N-acetyl-β-glucosaminidase	0	3	3	0	0
19	α-mannosidase	0	0	0	0	0
20	α-fucosidase	0	0	0	0	0

Note: Value in the column indicate the quantity of hydrolysed substrates: 0 = 0 nanomoles, 1 = 5 nanomoles, 2 = 10 nanomoles, 3 = 20 nanomoles, 4 = 30 nanomoles, 5 = >40 nanomoles

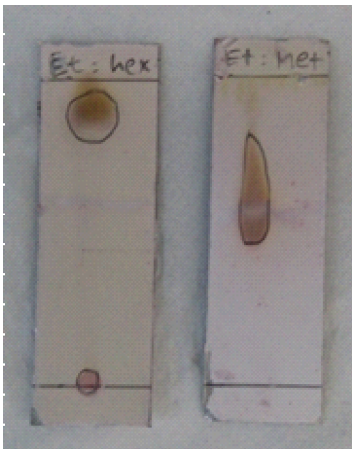
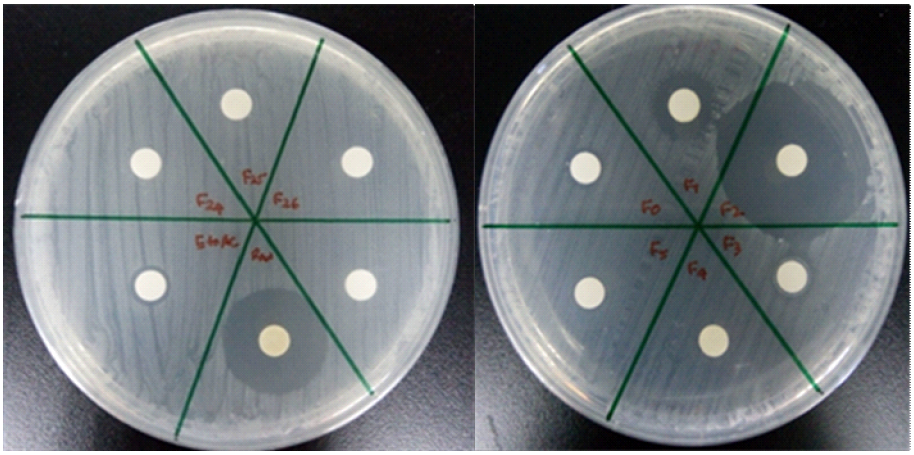


Figure 3. Performance of TLC for crude extract produced by MACMK-43 by several solvents. A (hexane: ethyl acetate); B (methanol : ethyl acetate).



A. Before fractionation B. After fractionation, Fraction-2

Figure 4. Clear zona of 2nd fraction of secondary metabolite produced by MACMK-43 strain.

substrates; and MACMK-80 strain which has very weak enzymatic activity compared to others even if it has strong antimicrobial ability.

The MACMK-43 strain showed positive reaction to the following enzymatic activity: alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, Naphthol-AS-BI-phosphohydrolase, b-glucosidase, esterase and N-acetyl-b-glucosaminidase, cystine arylamidase, a-chymotrypsin, but no enzymatic activity against a-galactosidase, b-galactosidase, b-glucuronidase, and a-glucosidase. Nearly all substrates that could be degraded by MACMK-43 showed a large quantity of >40 nanomoles. It can be suggested that this strain has a high enzymatic potential to develop.

The MACMK-80 strain showed poor positive reaction to the following enzymatic activity: alkaline phosphatase, esterase, esterase lipase, acid phosphatase, Naphthol-AS-BI-phosphohydrolase, and a-glucosidase. All substrates that could be degraded by MACMK-80 showed very low quantity in about 5 nanomoles. This evidence should be studied further because it is unique by its strong antimicrobial ability but weak enzymatic activity.

3.3. Isolation and Partial Purification of Active Compounds from MACMK-43 Strain

Hexane and ethyl acetate were used for fractionation of active compound extracts produced by MACMK-43. The selection of the solvent was based on the TLC results where the crude extract separated using hexane : ethyl acetate compared to methanol : ethyl acetate (Figure 3).

Fractionation of the MACMK-43 crude extract was performed by chromatographic columns and yielded as many as 26 fractions. The 26 fractions were

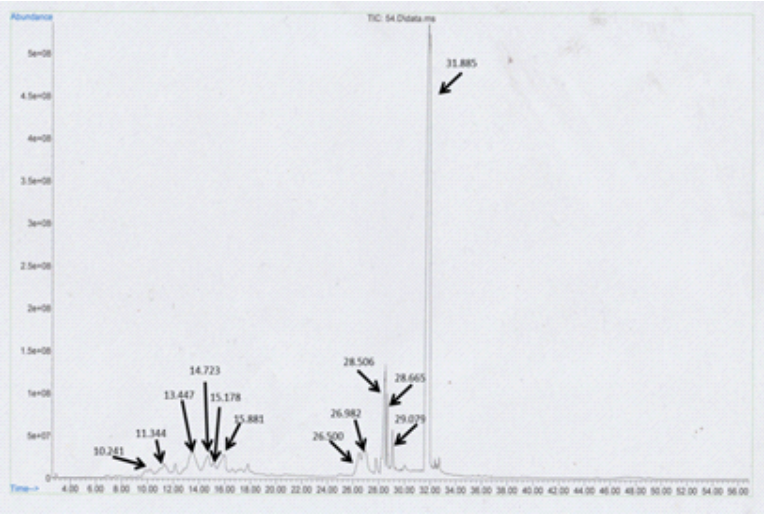


Figure 5. Chromatogram of 2nd fraction of active metabolite produced by *Streptomyces violascens* strain MACMK-43, analysed using Gas Chromatography Mass Spectrophotometer (7890B, Agilent).

Table 4. Metabolite profiling of 2nd fraction from secondary metabolite produced by MACMK-43 strain; analysed by GCMS and compared to library database in the tool

No	RT	Quality	Compound	Percentage (%)
1	10.241	38	Benzenemethanol, ar-ethyl	1.08
2	11.344	64	2-pyridinecarbonitril, 3-ethyl-1,2,5,6-tetrahydro-1methyl	1.47
3	13.447	55	1H-indole, 2,3-dihydro-1,2-dimethyl	6.44
4	14.723	96	2,4,6-trimethylbenzyl alcohol	3.38
5	15.178	93	Benzene, 1-ethenyl-4-methyl	1.2
6	15.881	60	4-methyl-1-indonone	2.79
7	26.5	97	Phenol, nonyl	2.94
8	26.907	87	Phenol, nonyl	2.59
9	26.982	93	Nonyl-phenol mix of isomers	1.57
10	28.506	99	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	7.25
11	28.665	72	Dibutyl phthalate	2.67
12	29.079	99	1-methyl[2,2]paracyclophan-1-en	1.59
13	31.885	90	4-amino-5-cyano-6-(4'methoxyphenyl)-1-methyl-2,3-dihydropyrrolo[2,3-B]pyridine	59.67

tested for resistance against *C. albicans*, and found that the most active compound was showed in the 2nd fraction (Figure 4). The active compound of the 2nd fraction (Figure 4B) strongly inhibited the growth of *C. albicans* compared to the raw compound before purification (Figure 4A). This indicates that the active compound has potential as antimicrobial, especially antifungal against *C. albicans*.

The chromatogram of GCMS examination is shown in Figure 5. It shows that the 2nd fraction of active metabolite contain 12 single peaks (Figure 5). Compared to the library database of the tool, the fraction is contained of compound as seen in Table 4. The highest peak is the compound in retention time (RT) of 31.885 minutes, with the largest content of 59.67% and the quality of 90%. The compound is suspected to be 4-amino-5-cyano-6-(4'methoxyphenyl) -1-methyl-2,3-dihydropyrrolo [2,3-B] pyridine.

Many researchers reported pyridine derivatives have varying abilities, such as N-(3, 5, 6-Trichloro-2-

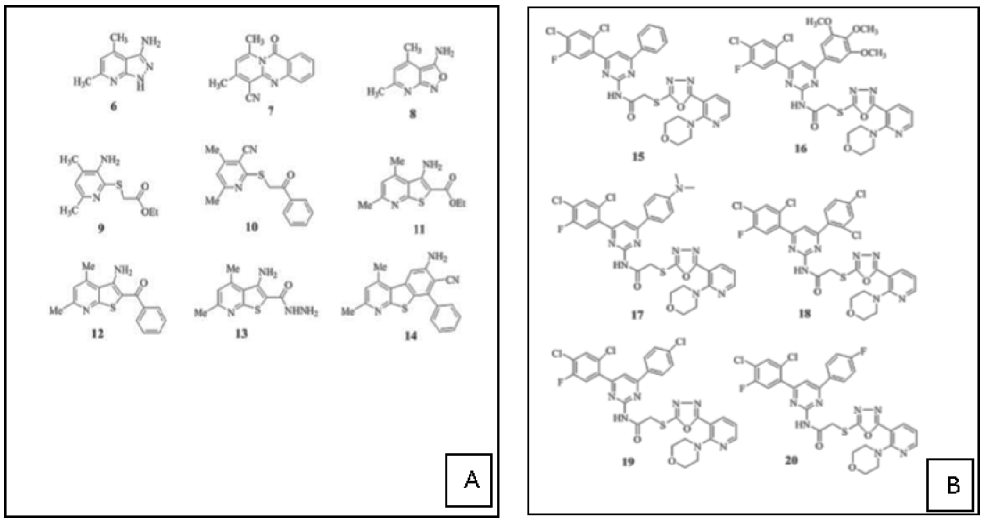


Figure 6. A. Representative examples of antimicrobial compounds containing pyridine as basic unit; B. Oxadiazole substituted Pyridine derivatives active against various bacteria (Altaf et al., 2015).

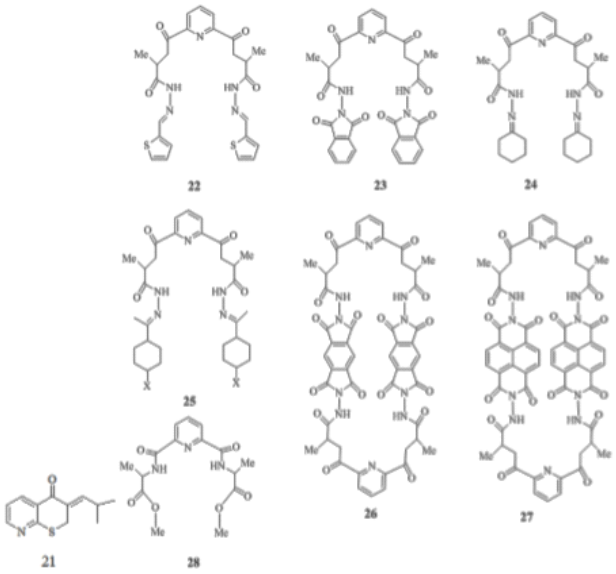


Figure 7. Oxadiazole substituted Pyridine derivatives active against various bacteria (Altaf et al., 2015).

pyridyloxyacetyl)N'-aroylhydrazines and N-(3,5,6-Trichloro-2-pyridyloxyacetyl)-N'arylsulfonylhydr-azines as an antibacterial activity against gram negative *Eschericia coli* and gram positive bacterium *Staphylococcus albus*, also as herbicides against *Cynodon dactylone, Cyperus rotundus, Echinochola crusgalli, Euphorbia hirta, Celocia argentia, Eugenia indica* and *Tridax procumbens* (Altaf et al., 2015), and as an antifungal agent against *Aspergillus niger* and *Aspergillus teniussiama* (Chavan et al., 2006). Octenidiene - a pyridine derivate which comprising hydrophilic and lipophilic parts - as an antimicrobial agent (Savchenko, Dorokhov, Yakushchenko, Zyuzin, & Aldoshin, 2010). Thienopyridine and other pyridine-derived compounds of type 6-14 (Figure 6A) as antibacterial agent against gram positive *S. aureus* and gram negative *E. coli, Pseudomonas aeruginosa* and *P. vulgaris* (Zav'yalova, Zubarev, & Shestopalov, 2009). The 3-hydroxypyridine-4-ones and 3-hydroxy-pyran-4-ones compounds active against *S. aureus, A. niger* and *C. albicans* (Sabet, Fassihi, & Moeinifard, 2009). Type of 3-substituted methylene-2H-thiopyrano pyridin-4 (3H) -ones [2,3-b] compound was tested as an antifungal in vitro (Altaf et al., 2015)

Figure 6B shows several types of pyridine derivatives. Oxadiazole substituted pyridine derivatives such as compounds of types 15 and 16 showed antimicrobial activity against *E. coli*, *S. aureus,*

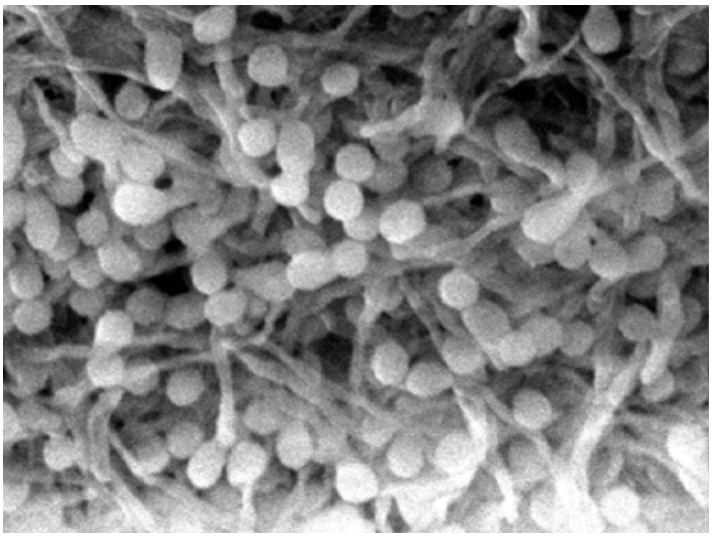


Figure 8. Photo Scanning Electronic Microscopy (JSM 5310 LV, JEOL) of MACMK-43 strain.

Salmonella typhi and *B. subtitlis*, the type 17 compounds have activity against *E. coli*, type 18 compound inhibited *S. aureus*, while compounds 19 and 20 were active against *S. typhi,* and type 20 compounds also exhibit activity against gram positive *B. subtilis* compared with standard drugs (Naik and Chikhalia, 2007).

Altaf et al. (2015) reported that compound 21 to 28 are macro-cyclic and open chain 2,6-substituted pyridine derivatives and also have many activities, such as activity against *Microsporum gypseum, Candida krusei,* and *Candida glabrata*, and some are almost proportional to the Ketaconazole or Ciprofloxacin (Al-Salahi, Al-Omar, & Amr, 2010).

As mention above that many of pyridine derivatives have varied abilities in biological application, such as antimicrobial, antibacterial, antifungal, herbicide, and others (Altaf et al., 2015). Therefore, this study potential to be developed so that the ability of MACMK-43 which has similarity of 97,85% with *Streptomyces violascens* can be further observed its ability to produce secondary metabolite, not only as antimicrobial agents.

3.4. The Profile of MACMK-43 Streptomyces Violascens Strain by Scanning Electron Microscopy (JSM 5310 LV, JEOL)

The profile of MACMK-43 strain under Scanning Electron Microscopy (JSM 5310 LV, JEOL) with MAG X10,000 condition; ACCV 20kV; WIDTH 13.2um is shown in Figure 8. The isolate has spores and mycelium that are the typical morphology of genus *Streptomyces*.

4. Conclusion

There were 36 strains of actinomycetes obtained from the deepsea sediments of Makassar Strait that have ability to produce antimicrobial compounds. Fourteen strains inhibited the growth of *E. coli*, 5 strains inhibited gram positive *S. aureus* and *B. subtilis*, and 27 strains inhibited *C. albicans*. The best strain is MACMK-43 which has 97.85% 16S rRNA gene sequence similarity with *Streptomyces violascens*. It produced secondary metabolite where the active fraction is suspected to be 4-amino-5-cyano-6-(4'methoxyphenyl)-1-methyl-2.3-dihydropyrrolo[2,3-B]pyridine which commercially used for bactericides and antihistamine.

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