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Anti-bacteria and toxicity potential of a rare Actinobacterium *Pseudonocardia* sp. SM1A, isolated from Mangrove Park, West Kalimantan, Indonesia

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Abstract. *Nofiani R, Rizky, Briliantoro R, Ardiningsih P. 2021. Anti-bacteria and toxicity potential of a rare Actinobacterium* Pseudonocardia *sp. SM1A, isolated from Mangrove Park, West Kalimantan, Indonesia. Biodiversitas 23: 453-458.* This study aimed to identify and evaluate antibacterial and toxicity activities of a rare Actinobacterium isolated from mangrove mud, Mempawah District, West Kalimantan. The mangrove mud sample was inoculated on ISP4 agar dissolved with seawater enriched meropenem (75µg/mL) and nystatin (100 µg/mL) using pour plate procedure and incubated at room temperature until the appearance powdery colony. One of the 2 powdery colonies successfully isolated was SM1A. SM1A was morphologically and biochemically identified to determine its genus and tested antibacterial activities using the cross streak method. SM1A also was cultivated and shaken at room temperature. After 10 days, the culture was extracted using ethyl acetate and then the extract was tested antibacterial activities using a well diffusion method and toxicity using brine shrimp lethality test (BSLT). SM1A was identified as *Pseudonocardia* sp., then was called *Pseudonocardia* sp. SM1A. *Pseudonocardia* sp. SM1A tested anti-bacteria using the cross streak method showed active against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, and *Streptococcus mutants* for the ISP4 agar medium and *S. typhi*, *S. mutants*, and *V. cholera* for the ISP1. *Pseudonocardia* sp. SM1A extract prepared from ISP1 broth medium was active against *S. typhi*, *E. coli*, *V. cholera*, and *Streptococcus mutants*. In addition, *Pseudonocardia* sp. SM1A extract cultivated on ISP1 broth medium showed LC_{50}=11.2570 µg/mL based on the BSLT and categorized as moderately toxic level. *Pseudonocardia* sp. SM1A contained secondary metabolites having high potential as lead compounds for drug discovery.

Keywords: Actinobacteria, antibacterial activities, *Pseudonocardia*, toxicity activity

INTRODUCTION

Cancer and infectious diseases are leading causes of death in the world. The number of cases of infectious diseases and cancer continues to increase every year. One of the reasons behind this increase in cases is the emergence of resistance to antibiotics and cancer or tumor drugs. Therefore, various secondary metabolite sources from different habitats are needed to obtain lead compounds for new antibiotics and cancer or tumor drugs.

The mangrove forest is one of the unique habitats due to the intersection between seawater and brackish water here, which causes different degrees of salinity (Ulumuddin and Setyawan 2017). The mangrove forest covers five districts in West Kalimantan, namely: Kubu Raya district, Sambas district, Mempawah district, North Kayong district, and Ketapang district. Microorganisms in the mangrove ecosystem generally develop an adaptation system that probably induces cryptic secondary metabolite biosynthetic gene clusters to produce novel secondary metabolites. Most secondary metabolites isolated from mangrove microorganisms exhibit unique structures such as secondary metabolites produced by Actinobacteria.

Actinobacteria are rich in secondary metabolites and become potential sources for drug discovery, notably *Streptomyces* sp. *Streptomyces* sp. GT2002/1503 isolated from mangrove produces Xiamycin with anti-HIV activity (Ding et al. 2010; Xu et al. 2014). *Streptomyces* sp. is an Actinobacteria genus with high-frequency isolation than other Actinobacteria genus. Most drugs in the market are from *Streptomyces* sp. High exploitation of *Streptomyces* sp. by researchers until now causes high probability re-isolation of known secondary metabolites. The researchers have started to explore the other Actinobacterial genus, for example, rare Actinobacteria such as *Thermomonospora*, *Actinoplanes*, *Microbispora*, *Thermoactinomyces*, *Streptosporangium*, *Micropolyspora*, *Pseudonocardia*, and *Microellobosporia* (Jafari et al. 2014). The frequency of isolation of the rare Actinobacteria usually is less than 0,5 %, while *Streptomyces* sp. shows the frequency isolation is almost 95% (Jafari et al. 2014). Therefore, the rare Actinobacteria show high potency to isolate the novel secondary metabolites with various biological activities.

This study was carried out to isolate Actinobacteria from the mud sample collected from mangrove park, Mempawah District, West Kalimantan. Then, a rare Actinobacterium was determined its genus and screened antibacterial and toxicity activities. This study aimed was to identify and evaluate antibacterial and toxicity activities of an Actinobacterium isolated from mangrove mud, Mempawah District, West Kalimantan, Indonesia.

MATERIALS AND METHODS

Materials

Media and other materials used in this study were ISP1 (International Streptomyces Project 1), ISP4 (International Streptomyces Project 4), NA (Nutrient Agar), LB (Luria-Bertani), phenol red base broth, sulphite indole motility (SIM), Simmons citrate agar (SCA), skim milk, corn starch, D(+)-glucose, D(+)-sucrose, D(-)-mannitol, and MR-VP broth. Chemical reagents used in this study were pro analysis grade, namely meropenem ($C_{17}H_{25}N_3O_5S$), nystatin ($C_{47}H_{74}NO_{17}$), tetracycline ($C_{22}H_{24}N_2O_8$), ethyl acetate ($C_4H_8O_2$), seawater, immersion oil, Gram-staining kit, NaCl, H_2O_2, tetramethyl-p-phenylenediamine dihydrochloride, methyl red, α-naphthol, KOH. *Artemia salina* eggs used in this study were Supreme Plus brand. The test bacteria used in this study were as follow: *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, and *Streptococcus mutants.*

Procedures

Sampling

The samples were taken at a depth of 15 cm with coordinate N 0º23'49.812" E 108º56'44.2068" from the Mangrove Park, Mempawah District, West Kalimantan, Indonesia in September 2019. The sample was transferred into sterile plastic and brought to the laboratory.

Isolation of actinobacteria

Sample suspension was prepared by mixing the mud sample (1 g) with 9 mL of sterilized distilled water using a vortex for 3x10 s. The sample suspension was allowed to stand to separate solid and liquid. One mL of the liquid sample mixed with warm ISP4A+SW (ISP4 agar dissolved with seawater) enriched meropenem (75µg/mL) and nystatin (100 µg/mL) then poured on a plate and incubated at room temperature. The appeared colonies on the medium surface were observed every day for up to a month. The suspected colonies, such as powdery or wrinkle or dull, were purified by transferring them to new ISP4A+SW to get pure isolates.

Characterization of actinobacteria

The morphological and biochemical features of Actinobacteria were evaluated following the procedure described by Li et al. (2016). The morphological features were observed either cellular (cell shape, spore, sporangia, sporangiospores) or colony (shape and size, color, dimensions, form, motility, diffusible and melanoid pigment). NaCl tolerance, pH tolerance, carbohydrate fermentation tests (D(+)-glucose, D(-)-mannitol, D(+)-sucrose fermentation tests), enzyme characteristic tests (protein hydrolysis test, starch hydrolysis, catalase test, oxidase test, *decarboxylate lysine* test), metabolite products (IMViC tests (indole test, methyl red test, Vorges-Proskaues test, citrate utilization test) and hydrogen sulfide (H_2S) production test) were used to characterize SM1A.

Spore Actinobacteria were examined for their morphology using the slide culture method. The spore was streaked on ISP1 agar media sized 5 mm^2, put on a sterile slide, covered with a sterile coverslip, and then incubated at room temperature. The growth of spores and mycelia was observed under a light microscope with 100x magnification on days 7, 14, 21, 28, and 30. The morphological cell wall Actinobacterium was also examined using Gram staining following the manufacturing guideline. Actinobacteria were streaked on various media (ISP1, ISP2, ISP3, and ISP4) and incubated for 3-21 days. After that, their spore color on the colony surface, diffusible and melanoid were observed. The diffusible pigment was exhibited its color on the reverse side colony and called distinctive (+) for red, yellow, green, blue, or violet and not distinctive (-) for pale yellow, olive, or yellowish-brown. Greenish brown, brownish-black on the medium surface was recorded as a positive melanoid pigment. Out of greenish-brown, brownish-black color on the medium surface was recorded as a negative melanoid pigment.

Actinobacteria were inoculated on ISP1 agar supplemented with various NaCl concentrations, namely: 0%, 2.5%, 5%, 7.5%, 10% and 12.5% then incubated at room temperature for 3-21 days. The colony growth was observed to evaluate NaCl tolerance. Actinobacteria were streaked on ISP1 agar with various pH (4, 5, 8, 9, 10, 11, and 12), incubated at room temperature for 3-12 days, and observed for colony growth.

The carbohydrate used for carbohydrate fermentation tests were D(+)-glucose, sucrose, and D(+)-mannitol. Each phenol red base broth was enriched with 1% D (+)-glucose, 1% sucrose, and 1% D(+)-mannitol, respectively then each tube was provided with a Durham tube. One inoculating loop Actinobacterium was inoculated into each tube and incubated at room temperature. After 3-4 days, red to yellow culture showed a positive test result, and yellow culture was concluded as a negative test result.

Actinobacteria were inoculated on 2 different media, namely ISP2 agar supplemented with 1% corn starch for starch hydrolysis test and ISP2 agar supplemented with 1% skim milk for protein hydrolysis tests. After 3-4 days of incubation at room temperature, the emergence of a clear zone around the colony showed positive for both the tests. One inoculating loop of Actinobacterium was inoculated on urea broth for the urease test. After incubation at room temperature for 3-4 days, the medium color changed from yellow to pink-red and was recorded as a positive urease test. For the catalase test, one inoculating loop of Actinobacterium was mixed with a drop of water on a slide, then added one or two drops 3% of hydrogen peroxide (H_2O_2). Without or with air bubbles showed negative or positive catalase tests, respectively. One inoculating loop of Actinobacterium was directly streaked on the wet Whatman's No. 1 filter paper piece for the oxidase test. The wet Whatman's No. 1 filter paper piece was prepared by dripping ten drops of fresh 1% tetramethyl-p-phenylenediamine dihydrochloride. A deep-purple hue in the media around the colony was recorded as a positive oxidase test. A lysine decarboxylase test was performed by streaking one inoculating loop on a lysine iron agar slant. After incubation at room temperature for 3-4 days, violet color on media indicated a positive result.

One inoculating loop of spore Actinobacterium was stabbed on SIM medium for motility and H_2S production tests then incubated at room temperature for 3-4 days. The positive motility was signed as a diffuse spreading growth around the colony, while positive H_2S production was showed black precipitate. Citrate utilization test, one inoculating loop of spore Actinobacterium was stabbed on SCA slant and incubated at room temperature for 3-4 days. A positive citrate utilization test was the medium change from green to blue.

Methyl red (MR) and Voges Proskauer (VP) tests were conducted simultaneously. Actinobacteria were inoculated into two tubes containing 3 mL of MR-VP broth medium. One tube was added with a drop of methyl red reagent (0.25 g of methyl red dissolved in 100 mL of ethanol) on day 4 of incubation time. Positive and weak positive MR tests were signed with red and red-orange culture, respectively, while negative MR test was showed with yellow or orange color. The other tube was added with 0.6 mL of reagent A (5.0 g of α-naphthol in 100 ml of absolute (100%) ethanol) and then mixed with 0.2 mL of reagent B (40.0 g of KOH in 100 ml of distilled water) for VP test. After incubation for 15-60 min, a pink-cherry red color developed at the surface of the medium was positive VP test while yellow color was for negative VP test.

Screening of antibacterial activities

Screening of antibacterial activities for Actinobacteria was carried out using cross streak. The test bacteria used in this assay were *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa*, *V. cholera*, and *S. mutants.* The cross streak method was carried out by inoculating isolate Actinobacterium forming a straight line in the middle of the media. After 6 days of incubation, each test bacteria were streaked in the left and right sides of the SM1A and incubated at room temperature for 3-5 days. A clear zone between isolate Actinobacterium and a bacterial test showed a positive antibacterial test. No clear zone indicated a negative antibacterial test. Furthermore, an inoculating loop was touched on the clear zone, then was streaked on a NA medium and incubated at room temperature for 12-16 hours. Appeared colonies on the NA medium surface were considered bacteriostatic activity. No colonies on the NA medium surface were recorded as bactericidal activity.

Production and extraction

Actinobacterial inoculum was prepared by inoculating 1 cm^2 of the medium containing spores into 20 mL of ISP1 broth and shaken at 200 rpm and room temperature. After 3 days, 2 mL of the seed culture was inoculated on 100 mL of ISP1 broth (Erlenmeyer 500 mL) and shaken at 200 rpm and room temperature for 10 days. Then, the culture was centrifuged at 1,048 x g for 40 min and obtained supernatant and cell. The supernatant was extracted using ethyl acetate with a ratio 1:1 for supernatant and ethyl acetate in triplicate. All of the organic layers were combined and evaporated using a rotary evaporator to gain the crude extract SMIP.

Antibacterial assay of the extract using well diffusion method

Antibacterial assay was conducted using the well diffusion method (Nofiani et al. 2020). The test bacteria used in this assay were *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa*, *V. cholera*, and *S. mutants.* Each test bacterium was cultivated on Luria-Bertani (LB) broth and shaken at 200 rpm, room temperature for 12-14 hours. Then, each bacterial test culture (1.000 µL) was mixed with 22.5 mL of warm LB agar media then poured on a petri dish with 10 cm diameter. After solid, well in the medium was perforated using a sterile puncher with 6 mm of diameter size. Each well was poured with the crude extract (700 µg/well), tetracycline (10 µg/well, positive control) and ethyl acetate (20 µL/well, negative control). After the solvent in the well evaporated, the petri dish was incubated at room temperature for 12-16 hours. The positive test was formed a clear zone around then the diameter of the clear zone was measured using a vernier caliper.

Toxicity assay using Brine Shrimp Lethality Test (BSLT)

BSLT was used to evaluate toxicity activity. BSLT was performed as described by Meyer et al. (1982). Hatching of brine shrimp eggs, *A. salina*, was conducted with seawater at room temperature for 2 days. The ten nauplii were collected by pipette and transferred in a small vial containing 700 µL of seawater then added 20 µL of the extract with various concentrations (200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6 µg/mL, 3.125 µg/mL and 0 µg/mL) then added seawater until 1 mL and incubated at room temperature for 24 hours. The dead nauplii were counted with the aid of a magnifying glass to obtain the percentage of death at each dose and control (without the crude extract) and were calculated as follows: % deaths=[(test-control)/control] x 100%. The test was carried out in a quadruplicate. Lethal concentration 50% (LC_{50}) was counted using probit analysis with IBM SPSS statistic 23.

RESULTS AND DISCUSSION

Isolation and characterization of actinobacteria

The mud sample was inoculated on ISP4 medium, incubated at room temperature, and observed for the growth of colonies every day for 2 months. The number of growth suspected colonies was two colonies. One of the two colonies was a dull and tough growing after 21 days of observation. This colony grew faster than the others. This colony was purified to obtain a pure colony and called SM1A. The colony produced white powdery spores at the top of the colony after 14 days of incubation. Hyphae SM1A was stained using the Gram staining kit and produced purple hyphae. It showed that SM1A was a Gram-positive bacterium strengthened as an Actinobacterium (Figure 1). SM1A also produced a white spore on ISP1 agar, ISP2 agar and ISP3 agar media (Table 1). SMIA generated yellow (ISP2 agar) and light red (ISP4 agar) on the reverse side colonies, which indicated distinctive for diffusible pigment test but no distinctive for

ISP1 and ISP2 agar media (Figure 1). SMIA did not produce melanoid pigment on ISP1, ISP2, ISP3, and ISP4 agar media.

Spore and aerial hyphae of isolate SM1A were observed using ISP1 agar with the slide culture method. Isolate SM1A produced a spore with an ovoid form at the end of aerial hyphae on day 28 (Figure 2). The aerial hyphae were branched and contained septa. *Psedonocardia* sp. usually was observed with septa on aerial hyphae. Some of them produce ovoid spores at the end of the hyphae. The hyphae and spore morphology of SM1A was similar to that of *Psedonocardia* sp. (Figure 2)(Holt et al. 1994; Lee et al. 2004; Low et al. 2015). Therefore, SM1A belonged to the Pseudonocardia genus and was named *Pseudonocardia* sp. SM1A.

Biochemical characteristics of isolates can usually be used to differentiate among *Pseudonocardia* species. *Pseudonocardia* sp. SM1A showed optimal growth when the medium did not contain NaCl but can grow to 7.5% of NaCl in the medium. pH range for *Pseudonocardia* sp. SM1A growth was 5-10, but optimal growth was pH 8. SM1A only ferment D(-)-mannitol, D(+)-glucose and sucrose. SM1A showed a positive catalase test, which general characteristic of Actinobacteria. The other enzyme tests (protease, amylase, urease, oxidase, and lysine decarboxylase), SM1A showed negative results. SM1A only showed a positive indole test result for IMViC tests.

Antibacterial activities

Antibacterial activities of *Pseudonocardia* sp. SM1A was evaluated using two methods, namely the cross streak and well diffusion methods, that differed in fermentation conditions. The cross streak method used solid-state fermentation (SSF), while in well diffusion method, liquid state fermentation (LSF) method was used. The cross streak method used two different media (ISP1 and ISP4) that were differed in composition of carbon and nitrogen sources. ISP1 medium used was high in nitrogen source from tryptone and yeast extract but very low carbon source while, ISP4 medium contained a high carbon source from starch but deficient in nitrogen. *Pseudonocardia* sp. SM1A showed antibacterial activities using the cross streak method. From this method, the antimicrobial compounds produced by *Pseudonocardia* sp. SM1A could be categorized as extracellular due to being secreted outside the cell. *Pseudonocardia* sp. SM1A showed antibacterial activities better on ISP4 agar medium than ISP1 agar medium. SM1A grew on ISP4 can inhibit 5 of six test bacteria, while on ISP1 only inhibit 3 of 6 test bacteria (Table 1). The high carbon contained in the ISP4A+SW medium might increase the titer or type of antibacterial compounds produced by *Pseudonocardia* sp. SM1A. The carbon source increase antibacterial production of microorganisms such as *Bacillus megaterium* NB-3, *Bacillus cereus* NB-4 (El-Banna 2005), and *Rhodobacteracea bacterium* SP.2.11 (Murniasih and Bayu 2015). The media affecting the secondary metabolites have been reported by many scientists (Kumar et al. 2014; Benhadj et al. 2019; Nofiani et al. 2020; Al-Ansari et al. 2020). Antibacterial activities of *Pseudoocardia* sp. SM1A on both the media using the cross streak method showed bacteriostatic activity except for *S. typhi* on ISP1 that acted as bactericidal activity. An agent inhibiting or killing the growth of test bacteria is called bacteriostatic or bactericidal activity (Rhee and Gardiner 2004).

Table 1. Morphological and biochemical characteristics of isolate SM1A

Tests	Results
Morphology	
Gram staining	Positive
Motility	-
Spore mass color	
ISP1	White
ISP2	White
ISP3	White
ISP4	White
Diffusible pigment	
ISP1	-
ISP2	d, yellow
ISP3	-
ISP4	d, red
Melanoid pigmentation	
ISP1	-
ISP2	-
ISP3	-
ISP4	-
NaCl tolerance	
0% NaCl	+
2.5% (w/v) NaCl	+
5% (w/v) NaCl	+
7.5% (w/v) NaCl	±
10% (w/v) NaCl	-
12.5% (w/v) NaCl	-
NaCl optimum	0%
pH Tolerance	
pH 4.0	-
pH 5.0	+
pH 8.0	+
pH 9.0	+
pH 10.0	+
pH 11.0	-
pH 12.0	-
Carbohydrate fermentations	
D(+)-glucose	+
D(+)-Mannitol	+
Sucrose	+
Enzyme characteristic tests	
Protease	-
Amylase	-
Urease	-
Catalase	+
Oxidase	-
Lysine decarboxylase	-
Metabolic products	
Indol	+
Methyl red	-
Voges Proskauer	-
Simon citrate	-
H_2S production	-

Note: +: positive test/growth;-: negative test/growth. ±. less growth. d: distinctive. nd: not distinctive. Sign for melanoid pigment: +: produced pigment;-: no produced pigment

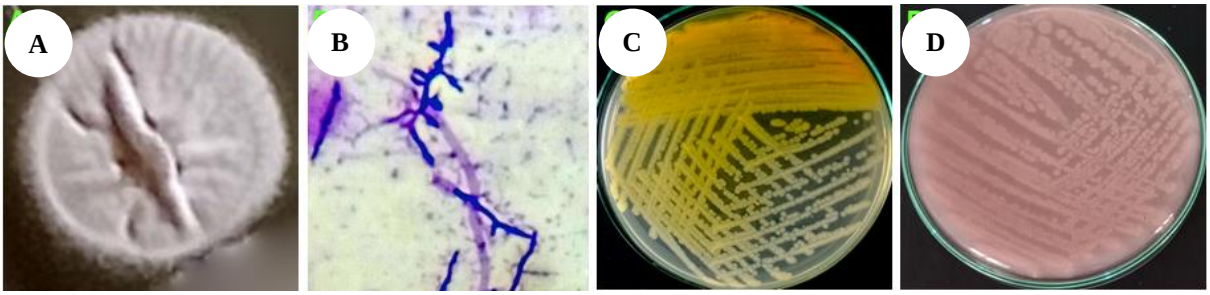

Figure 1. Morphological colony and hyphae of isolate SM1A. A. Mycelial colony SM1A covered by white spore on ISP1 agar; B. Branched hyphae SM1A stained with the Gram staining; C. Distinctive diffusible pigment SM1A on ISP2 agar. D. Distinctive diffusible pigment SM1A on ISP4 agar

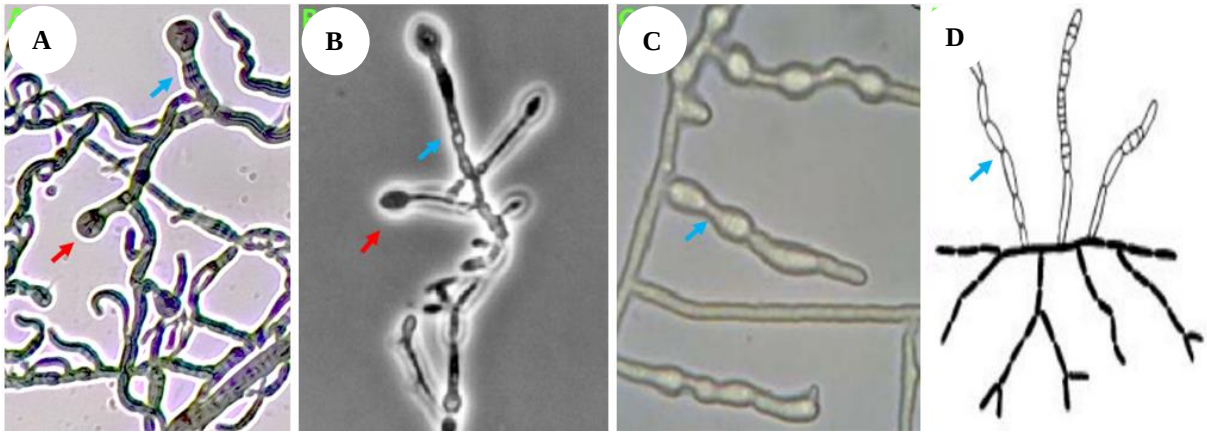

Figure 2. Hyphae (blue arrow) and spore formation at the ends of hyphae (red arrow). A. SMIA has grown on ISP1 agar for 28 days at room temperature and observed with 100x magnification; B. *Pseudonocardia chloroethenivorans* SL-1 (Lee et al. 2004); C. *Pseudonocardia* sp. (Low et al. 2015) D. *Pseudonocardia* sp. (Holt et al. 1994)

Table 2. Antibacterial activities of Pseudonocardia sp. SM1A

Test bacteria	Gram bacteria	Method of antibacterial test			
		Cross streak		Dosis µg/well	Diameter of inhibition zone (mm) for well diffusion
		ISP1	ISP4		ISP1
Staphylococcus aureus	Positive	-	+, Bs	750	-
Streptococcus mutants	Positive	+, Bc	+, Bs	750	1.6
Salmonella typhi	Negative	+, Bs	+, Bs	750	0.5
Escherichia coli	Negative	-	+, Bs	750	1.9
Pseudomonas aeruginosa	Negative	-	-	750	-
Vibrio cholerae	Negative	+Bs	+, Bs	750	1.3

Note: +. Inhibition zone.-. No inhibition zone. Bs. Bacteriostatic action. Bc. Bactericidal action.r=2 for the cross streak method.

Antibacterial activities tested using well diffusion methods showed that extract *Pseudonocardia* sp. SM1A prepared by fermentation with ISP1B+SW (ISP1 broth+seawater) was active against 4 of 6 test bacteria (Table 2). However, *Pseudonocardia* sp. SM1A showed different antimicrobial activities in the same media (ISP1) but different state fermentations (Table 2). The state fermentation probably changed the secondary metabolites profiles, particularly having antibacterial compounds. *Nocardia alba* FA9 fermented in the SSF is higher in the inhibition zone than the LSF but the same against the number of test bacteria (Salim et al. 2017). *Acremonium chrysogenum* produces higher cephalosporin-C production in the SSF than the LSF (Tabaraie et al. 2012).

Toxicity test using Brine Shrimp Lethality Test (BSLT)

Toxicity activity of *Pseudonocardia* sp. SM1A was evaluated using BSLT. The BSLT result can be correlated with anti-tumor, cytotoxic and pesticide activities (Ullah et al. 2013; Omeke et al. 2018). Probit model of *Pseudonocardia* sp. SM1A was y=0.058x-0.648. LC_{50}=11.2570 µg/mL. LC50 value can be used to define

the toxicity level of compounds (Meyer et al. 1982; Tanamatayarat 2016; Meena et al. 2020). Meyer et al. classifies toxic or non toxic for LC_{50} > 1000 µg/mL (non-toxic) and highly toxic for LC_{50} < 1000 µg/mL (Meyer et al. 1982); Tanamatayarat classifies toxicity level as follows: highly toxic for LC_{50} < 10 mg/mL, moderately toxic for LC_{50}: 10–100 mg/mL, weakly toxic for LC_{50}: 100– 1000 mg/mL), and inactive for LC_{50} > 1000 mg/mL (Tanamatayarat 2016). Clarkson classifies toxicity level as follows: no toxic for LC_{50} above 1000 µg/mL, low toxic for LC_{50} 500-1000 µg/mL, medium toxic for LC_{50} 100-500 µg/mL, and highly toxic for LC_{50} 0-100 µg/ (Meena et al. 2020). LC_{50} value of *Pseudonocardia* sp. SM1A is higher than that of ethanol extract *Streptomyces* sp. AJ4 (LC_{50} value =653.381 µg/mL, Rahayu et al. 2019), n-butanol extract *Streptomyces* sp. (LC_{50} value = 42.11 µg/mL, Prasith et al. 2010), and the metabolite purified from *Streptomyces* sp. CAS72 (LC_{50} value =23.5 µg/mL, Palaniappan et al. 2013). *Pseudonocardia* sp. SM1A extract was categorized as moderately toxic level based on LC_{50} that was classified by Tanamatayarat (2016).

In conclusion, *Pseudonocardia* sp. SM1A was successfully isolated from Mangrove Park, Mempawah District, West Kalimantan. *Pseudonocardia* sp. SM1A is a potential source of lead compounds for drug discovery, particularly antibacterial or toxicity activities. The toxicity activity of *Pseudonocardia* sp. SM1A probably has antitumor, cytotoxic, and pesticide activities.

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