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Antibacterial Effect of *Nocadia* sp. Against *Methicillin Resistant Staphylococcus aureus* (MRSA)

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ABSTRACT

Pathogenic bacterial resistance against antibiotic was one of major problem in medical concern. Staphylococcus aureus or Methicillin Resistant Staphylococcus aureus (MRSA) is one of those pathogenic bacteria. This research purposes to discover the new antibacterial substance using strain indentified as Nocardia sp. which belongs to Actinomycetes. Bacterial isolate was collected from Udayana University Microbiology Laboratory, Indonesia. Antagonistic evaluation using pour plate method in Nutrient Agar (NA) medium shows Nocardia sp. 1 strain has the highest result of inhibition zone test (22.7 mm long) against MRSA compared to 6 other strain. Nocardia sp. 1 strain filtrate tested on MRSA has a MIC level of 2%. Later identification of chemicals substance contained in Nocardia sp. 1 strain filtrate detects substances such as 3,5 -Dichloro- 2 pyridone, Benzeneacetic acid, Hepatdecane, Phenol. 2.4-bis(1.1-dimethylethyl), Dodecanoic Acid Methyl Ester, Hexadecanoic Acid Methyl Ester, 1-(+)-Ascorbic Acid 2.6 Dihexadecanoate.

Key words: *Antibacterial, Actinomycetes, Methicillin Resistant Staphylococcus aureus, MIC.*

INTRODUCTION

Methicillin Resistance Staphylococcus aureus (MRSA) is a Gram positive bacteria which is resistant against antibiotics, such as penicillin, oxacylin, beta-lactam, etc. In Asia region, *S. aureus* resistance to ciprofloxacin reaches 37%. Mardiastuti *et al.*,[10] reported that the percentage of Asia MRSA resistance is high in several countries like Taiwan 60%, China 20%, Hong Kong 70%, Philippine 5%, and Singapore 60%. *S. aureus* can cause many syndromes such as bacteremia, respiratory tract infection, endocarditic, urinary tract infection, and skin infection[4]. Based on that, it is important to find new antimicrobial substances, aside from the basic usage of antibiotic. *Actinomycetes* is the biggest source of microbes which produce secondary metabolites for instance antibiotic and nonantibiotic bioactive metabolites like enzymes and immunology regulator[6]. As *Actinomycetes* member, *Nocardia* sp. is able to produce secondary metabolites. Ruiz *et al.*[14] explains that Nocardicin A and B (*Nocardia* sp.), rifamycin (*Nocardia mediterranea*), ansamitocin (*Nocardia brasiliensis*), 3′-*O*-demethyl mutactimycin (*Nocardia transvalensis*), neo-nocardin (*Nocardia kuroishi*), and cephamycin C (*Nocardia lactamdurans*) were reported belong to secondary metabolites from genus Nocardia. Therefore, it is aimed in the present study firstly to investigate the capability of *Nocardia* sp. isolates in inhibiting MRSA growth, secondly to investigate the filtrate potential against MRSA, and the last is to determinate the Minimum Inhibitory Concentration (MIC) value.

MATERIALS AND METHODS

Samples Collection

Isolates culture of *Nocardia* sp. were collected from Udayana University Microbiology Laboratory, Indonesia. The numbers of *Nocardia* sp. strain being used are six isolates.

Antagonistic test of *Nocardia* sp. against *Methicillin Resistance Staphylococcus aureus* (MRSA)

Pour plate method was conducted for antagonistic test. Bacterial colony to be tested was planted in NaCl *physiology liquid* 0.9%. *Mc Farland* standard was (in 1.0 x 10^8 of bacterial density) used to uniform the bacterial turbidity. Then this bacteria was homogenized in *Nutrient Agar* (NA) medium in petridish. After it was hardened, *Nocardia* sp. was placed on the center of the petridish. Next it was incubated in 28±2°C for 24 hour[11].

Preparation of *Nocardia* sp. filtrate

The most potential *Nocardia* sp. that showed best inhibition zone was grown in *Yeast Extract Malt Broth* (YEMA) and incubated in 28±2°C for 24 hour. The grown *Nocardia* sp. strain was taken by using *cork borer* and placed inside of a glass bottle filled with YEMA, followed by incubation in a shaker at 80 rpm for 7 days[13]. Afterwards, 15 minutes centrifugation at 10^4 rpm was conducted, the resulted filtrate was evaporated then separated using ethyl-acetate 1:1 (v/v), then homogenized and settled for 24 hour. The next step is the ethyl-acetate phase was vaporized by evaporator machine at 40^{o}C. The resulted product was tested *in vitro* against MRSA using diffusion chamber method[13].

Minimum Inhibitory Concentration filtrate *Nocardia* sp. Using diffusion chamber method

Petridish filled with 10 mL NA and 200 µL MRSA isolate suspension was settled until it is hardened, then the center was holed with 5 mm *cork borer* to create a diffusion chamber. The chamber was filled with 20 µL *Nocardia* sp. filtrate at percentage (v/v) of 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and ethyl-acetate was used as negative control and linezolid as positive control. Each of concentration used triple repetition. The result was noted by measuring the inhibition zone.

Chemical compound analysis using Gas Chromatography Mass Spectrometry (GCMS) for *Nocardia* sp. filtrate

The highest inhibition zone test resulted on each phase between water phase and ethyl acetate phase undergo *Thin Layer Chromatography* (TLC) test. Filtrate was spotted on TLC plate (silica gelplat Merck 60 F254) which soluble with chloroform:ethyl-acetate:acetate-acid mixture (7:3:1, v/v). Active spot was visualized under UV rays λ254 and λ365 nm (Ali, 2009)[1]. Colum Chromatography was used to purify the compound and the identification of the filtrate compound was conducted using GCMS.

RESULT

Antagonist Activity of *Nocardia* sp. against *Methicillin Resistance Staphylococcus aureus* (MRSA)

Table 1. Inhibition zone of *Nocardia* sp. isolates against *Methicillin Resistance Staphylococcus aureus* (MRSA)

Number	Isolat *Nocardia* sp.	Inhibition Zone Diameter (mm)
1	*Nocardia* sp.1	22.7± 3.6
2	*Nocardia* sp. 2	14.7 ±3.3
3	*Nocardia* sp. 3	0.0±0.0
4	*Nocardia* sp. 4	7.3±5.6
5	*Nocardia* sp. 5	0.0 ±0.0
6	*Nocardia* sp. 6	0.0±0.0
7	Control	0.0±0.0

*Control: without *Nocardia* sp. isolate

Minimum Inhibitory Concentration (MIC) Filtrate *Nocardia* sp.1 against *Methicillin Resistance Staphylococcus Aureus* (MRSA).

Filtrate of *Nocardia* sp. 1 was tested for its ability to inhibit the growth of MRSA. In concentration of 100%, *Nocardia* sp.1 showed a diameter of 35.2 mm wide of inhibition zone while the positive control

using linezolid reached a diameter of 39.0 mm. *Nocardia* sp.1 filtrate started showing its ability in 10% concentration with a diameter of 11.6 mm. Afterwards, filtrate concentration was decreased untill 1% and it still showed an inhibition ability at 2% concentration.

Analysis of chemical compounds from *Nocardia* sp.1 filtrate using Gas Chromatography Mass Spectrometry (GCMS)

Chromatogram analysis of *Nocardia* sp.1 filtrate using Gas Chromatography Mass Spectrometry (GCMS-QP2010S SHIMADZU) displayed eight peak which can be observed in Table 2.

Table 2. Chromatogram of *Nocardia* sp. 1 chemichal substances profile starting from each peak

No	Peak	MW (Molecular Weight)	FM (Formula Mass)	Retension Time (minute)	Profile of Chemichal Substance base on MS database
1	Peak 1	163	$C_5H_3Cl_2NO$	7,564	3,5 -Dichloro- 2 pyridone
2	Peak 2	136	$C_8H_8O_2$	9,093	Benzeneacetic acid
3	Peak 3	240	$C_{17}H_{36}$	10,978	Heptadecane
4	Peak 4	240	$C_{17}H_{36}$	11,322	Heptadecane
5	Peak 5	206	$C_{14}H_{22}O$	12,703	Phenol. 2.4-bis(1.1-dimethylethyl)
6	Peak 6	214	$C_{13}H_{26}O_2$	12,902	Dodecanoic Acid Methyl Ester
7	Peak 7	270	$C_{17}H_{34}O_2$	17,412	Hexadecanoic Acid Methyl Ester
8	Peak 8	652	$C_{38}H_{68}O_8$	17,733	1-(+)-Ascorbic Acid 2.6 Dihexadecanoate

DISCUSSION

In this study, *Actinomycetes* indentified as Nocardia sp. 1 strain was found to produce compound showing antagonistic effect against MRSA by inhibition zone value with diameter of 22.7 mm. This substance can be primer metabolite and secondary metabolite. According to Ambavane *et al.*[2], the mechanism of secondary metabolite of *Actinomycetes* occurs by damaging cell wall and obstructing cell division. Singh *et al.*[15] research explains that several *Actinomycetes* isolated from different ground are proven to impede pathogenic resistance bacteria such as *Escherichia coli* and *Vancomycin-Resistant Enterococci* with 13 – 14 mm diameter of inhibition zone. Chau *et al.*[5] reported that *Actinomycetes* can inhibit *Vibrio* sp. by producing *siderophore* and extracellular enzymes.

The data obtained show *Nocardia* sp.1 filtrate has MIC level at 2% (v/v). Wardani *et al.*[17] reported that MIC level from *Nocardia* sp. extracted with n-butanole, ethyl-acetate and chloroform and tested against *Microsporum gypseum* and *Staphylococcus aureus* are 64 ppm and 128 ppm respectively. El-Gendy *et al.* (2008)[7] showed a result that a strain of *Nocardia* sp. ALAA2000 tested with Gram positive and Gram negative bacteria presented MIC level at 0.1 – 10 µg/mL.

The result of GCMS characterization analysis indicated several substances namely 3,5 -Dichloro- 2 pyridone, Benzeneacetic acid, Heptadecane, Phenol. 2.4-bis(1.1-dimethylethyl), Dodecanoic Acid Methyl Ester, Hexadecanoic Acid Methyl Ester, 1-(+)-Ascorbic Acid 2.6 Dihexadecanoate. Benzeneatisc acid was also reported that it has been isolated from *Streptomyces galbus* TP2 and *Streptomyces humidus* and it is known as antifungal and important substance in medical field[3,8]. This Benzeneatisc acid is expected as precursor during *Penicillium chrysogenum* fermentation to produce antibiotic penicillin G[12]. Whereas *Dodecanoic acid methyl ester* and *hexadecanoic acid methyl ester* were common substance synthesized by *Actinomycetes*. Suzuki[16] says that one indicator of *Actinomycetes* group classification is based on the existence of those substances. *Hexadecanoic acid methyl ester* were reported to be found specifically in *Nocardia levis* MK-VL-113 strain and has an antibacterial activity[9].

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REFERENCES

1. Ali, A., Skrining dan Krakterisasi Parsial Senyawa Antifungi dari *Actinomycetes* Asal Limbah Padat Sagu Terdekomposisi.*Berk. Penel. Hayati* **14:** 219–225 (2009)
2. Ambavane, V., Tokdar, P., Parab, R., Sreekumar, E.S., Mahajan,G., Mishra, P.D., D'Souza,L., Ranadive, P., Caerulomycin A-An Antifungal Compound Isolated from Marine *Actinomycetes*. Advances in Microbiology **4:** 567-578 (2014)
3. Andayani, D.G.S., Sukandar, E.Y., Sukandar ,U., Adnyana, I.K., Isolation, Identification of Phenylacetic Acid from Streptomyces galbus TP2 Strain and Its Toxicity. *Int j pharm pharm sci*. **6(5):** 643-646 (2014)
4. Bradley, S.F., *Staphylococcus aureus* Infections and Antibiotic Resistance in Older Adults. *Clinical Infectious Diseases*, 211–216 (2002)
5. Chau, T.T., Hieu, Nguyen.X., Thuan, Le.T.N.,Matsumoto,M., Mijayajima, I., Identification and Characterization of *Actinomycetes* Antagonistic to Pathogenic *Vibrio* spp. Isolated from Shrimp Culture Pond Sediments in Thua Thien Hue–Viet Nam. *J. Fac. Agr., Kyushu Univ*. **56(1):** 15–22 (2011)
6. Chaudhary, H., Soni B., Rawat Shrivastava A., Shrivastava, S., Diversity and Versatility of *Actinomycetes* and its Role in AntibioticProduction. *Journal of Applied Pharmaceutical Science* **3(8):** 83-94 (2013)
7. El-Gendy, M.M. A., Hawas, U.W., Jaspars, M. 2008. Novel Bioactive Metabolites from a Marine Derived Bacterium *Nocardia* sp. ALAA, *J. Antibiot.* **61(6):** 379–386 (2000)
8. Hwang, B.K., Lim, S.W., Kim, B.S., Lee, J.Y., Moon, S.S., Isolation and In Vivo and In Vitro Antifungal Activity of Phenylacetic Acid and Sodium Phenylacetate from *Streptomyces humidus*. *Applied And Environmental Microbiology* **67(8):** 3739–3745 (2001)
9. Kavitha, A., Prabhakar, P., Vijayalakshmi, M., Venkateswarlu, Y., Production of Bioactive Metabolites by Nocardia levis MK-VL_113. *The Society for Applied Microbiology, Letters in Applied Microbiology* **49:** 484–490 (2009)
10. Mardiastuti, H.W, Karuniawati, A. , Kiranasari, A., Ikaningsih, Kadarsih, R., *Emerging Resistance Pathogen*: Situasi Terkini di Asia, Eropa, Amerika Serikat, Timur Tengah dan Indonesia. *Majalah Kedokteran Indonesia* **57(3):** 75-79 (2007)
11. Muharram, M., Abdelkader, M., Alqasuomi, S., Antimicrobial Activity Of Soil *Actinomycetes* Isolated From Alkharj, Ksa. *International Research Journal of Microbiology* **4(1):** 12-20 (2013)
12. Pramisandi, A., Sunaryanto,R., Suyanto, Prabandari, E.E., Effect Of Phenylacetic Acid Addition On Productivity Of *Penicillium chrysogenum* In Penicillin G Production Using Pilot Scale Reactor. *Proceeding of International Conference on Chemical and Material Engineering*. Department of Chemical Engineering Diponegoro University (2012)
13. Rana, S. and Salam, M. D., Antimicrobial Potential of *Actinomycetes* Isolated from Soil Samples of Punjab, India. *Journal of Microbiology and Experimentation* **1(2):** 1-6 (2014)
14. Ruiz, B., Chávez, A., Forero,A., García-Huante,Y., Romero,A., Sánchez,M., Rocha,D., Sánchez,B., Rodríguez-Sanoja,R., Sánchez,S., Langley, E., Production of Microbial Secondary Metabolites: Regulation by The Carbon Source. *Critical Reviews in Microbiology* **36(2):** 146–167 (2010)
15. Singh, S., Kumar, P., Gopalan, N., Shrivastava, B., Kuhad, R.C., Chaundary, H.S., Isolation and Partial Characterization Of *Actinomycetes* With Antimicrobial Activity Against Multidrug Resistant Bacteria. *Asian Pacific Journal of Tropical Biomedicine* 147-150 (2012)
16. Susuki, K., Cellular Fatty Acid Analysis In Actinomycete Taxonomy. *Proccedings of Seventh International Symposium on Biology of Actinomycetes.* Japan (1988)
17. Wardani, I. G. A. A. K. , Andayani,d. G. S., Sukandar,U., Sukandar, E.Y., Adnyana, I.K., Study On Antimicrobial Activity Of *Nocardia* sp. Strain TP1 Isolated From Tangkuban Perahu Soil, West Java, Indonesia. *International Journal Of Pharmacy And Pharmaceutical Sciences* **5(2):** 713-716 (2013)