BIOEDUSCIENCE
ISSN: 2614-1558
http://journal.uhamka.ac.id/index.php/bioeduscience

Extraction and Identification Potent Antibacterial Bioactive Compound of Streptomyces sp. MB 106 from Euphorbia sp. Rhizosphere

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Abstract

Background: *Actinomycetes* are groups of bacteria that play an important role in pharmacy and medicine. They can produce secondary metabolites in bioactive compounds with various chemical structures and biological activities. The genus *Streptomyces* produces more than 70% of all described *actinomycetes* natural products. In particular, antibiotics represent the largest group. Through mutations, bacteria may survive chemotherapy. Thus its infection is hard to eradicate. Therefore the search for new potent bioactive compounds is important. **Methods:** Isolate preparation, Production, and Extraction of the bioactive compound, Crude extract antibacterial test, Identification of bioactive compound. **Results:** There are antibacterial activities per 100 µl crude extract—inhibition zone ranging from 11.5 to 13 mm. Various bioactive compounds detected in n-Butanol extract, There are nine bioactive compounds detected from *Streptomyces* sp. MB 106 crude extract. The highest was Naphthalene (43.89 %). The lowest was 3-Imino-3h,5h- [1,2,4] Thiadiazolo [3,4-1] Isoindole (1.70 %). Six compounds were reported to have antimicrobial activities butane, 1,1-dibutoxy, naphthalene, nonadecane, docosane, heneicosane, and eicosane. **Conclusions:** Crude extract showed an average of 12 mm inhibition zone against *Escherichia coli* ATCC 8739. There are nine bioactive compounds from *Streptomyces* sp. MB 106. Six of them are promising drugs candidate. Further studies on these compounds are essential for future drugs candidate.

Keywords: antimicrobial; euphorbia sp.; Extraction; rhizosphere streptomyces MB 106.

Article history
Received: 19 Nov 2021
Accepted: 28 Feb 2022
Published: 30 Apr 2022

Publisher's Note:
BIOEDUSCIENCE stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Citation:
Wijayanti, D.R., Dewi, A.P. 2022. Extraction and Identification Potent Antibaterial Bioactive Compound of *Streptomyces* sp. MB 106 from *Euphorbia* sp. Rhizosphere. *BIOEDUSCIENCE*, 6(1), 84-88.
Doi: 10.22236/j.bes/617898

Introduction

Microorganisms are fascinating natural sources of secondary metabolites, also known as bioactive compounds. Microbial bioactive compounds benefit human health. (Janardhan et al., 2014). Among commercially important producers of bioactive compounds, *actinomycetes* can produce enormous varieties of secondary metabolites. At least 23,000 bioactive secondary metabolites from microbes have been reported, of which 10,000 of them have been produced by *actinomycetes* (Rashad et al., 2015).

Actinomycetes are a group of bacteria that play an important role in pharmacy and medicine in their ability to produce secondary metabolites in bioactive compounds with various chemical structures and biological activities. Thousands of bioactive secondary metabolites have been isolated and characterized, many of which have been developed into medicines to treat various diseases in humans and animals (Janardhan et al., 2014). Eicosane (C20H42) and dibutyl phthalate (C16H22O4) were antifungal compounds produced by Streptomyces strain KX852460 (Ahsan et al., 2017). A bioactive compound

(Z)-1-((1-hydroxypenta-2,4-dien-1-yl)oxy) anthracene-9,10-dione with antioxidant activity was produced *by Nocardiopsis alba* isolated from mangrove soils (Janardhan et al., 2014). The genus Streptomyces produces more than 70% of all described *actinomycetes* natural products. In particular, antibiotics represent the largest group (Rashad et al., 2015; Zotchev, 2012). Twenty-two antibiotic bioactive compounds were isolated from *Streptomyces anulatus* NEAE-94. Docosane, eicosane, n-heneicosane, and nonadecane were some of them (El-Naggar et al., 2017)

The continued increase of pathogenic microorganisms against commercially known drugs underlines the urgent need for novel antimicrobial compounds. Through mutations, bacteria may survive chemotherapy. Thus its infection is hard to eradicate (De Simeis & Serra, 2021). Therefore the search for new potent bioactive compounds is important.

Our research used previously isolated *Streptomyces* species from *Euphorbia* sp. *Euphorbia* sp. is one of many succulent plants in Indonesia. Report on *actinomycetes* bioactive compound producer from its rhizosphere is scarce (Zulaika et al., 2021). This study extracted bioactive compounds from a *streptomyces* species. Bioactive compounds were tested against pathogenic bacteria and identified.

Methods

Isolate preparation

Streptomyces sp. MB106 was isolated from *Euphorbia* sp. Rhizosphere at Melrimba Garden, Bogor, West Java (Zulaika et al., 2021). This isolate was recovered from stock culture and inoculated on *Starch Casein Nitrate Agar* (SCNA), incubated for seven days at 28°C to 30°C (Sholkamy et al., 2020).

Production and Extraction of bioactive compound

Isolate on SCNA inoculated to 50 ml starter broth Starch Casein Nitrate Broth (SCNB) incubated in shaker 120 rpm for five days at 30 °C. Five ml of starter inoculated to the fermentation broth. Afterward, the fermentation broth was incubated at 30 °C, 120 rpm for seven days (pH 7.0). Culture filtrates were collected to extract bioactive compounds (Sholkamy et al., 2020).

Culture filtrate is extracted with n-Butanol (Kawuri & Darmayasa, 2019). Culture filtrate centrifuge at 10,000 rpm, supernatant obtained and separated. The solvent was added to the supernatant in 1:1 (v:v), followed by shaking in a separating funnel, and settled at room temperature for 15 minutes. Afterward continued with the evaporation process at 40°C. The crude extract was then tested for antibacterial activity.

Antibacterial test of bioactive compound

100 µl crude extract was tested for antimicrobial activity using the good diffusion method on the Nutrient Agar triple plate (Khattab et al., 2016). *Escherichia coli* ATCC 8739 was used to test antibacterial activity.

E. *coli* ATCC 8739 inoculated onto Nutrient agar. After full growth, wells were made by a cork borer. 100 µl crude extract was transferred into the well. n–Butanol and Streptomycin sulfate were used as control. Plates were incubated at 37°C. The diameter of the inhibition zone was measured after 48 hours of incubation.

Identification of bioactive compound

Bioactive compounds were identified by using Gas chromatography-mass Spectrometer (GC-MS). Column temperature was 80°C to 250°C. The temperatures in the injector and detector were respectively 250°C and 220°C. (Kawuri & Darmayasa, 2019).

Results

A bioactive compound antibacterial test showed a positive antibacterial activity *Streptomyces* sp. MB 106 100 µl of crude extract (Figure 1). The inhibition zone range from 11.5 to 13 mm, mean of 12 mm, as shown in Table 1.

Various bioactive compounds were detected in n-Butanol extract. There are nine bioactive compounds detected from *Streptomyces* sp. MB 106 crude extract (Table 2). The highest was Naphthalene (43.89 %), Heneicosane (22,51%) and Butane, 1,1-Dibutoxy (18,99%). The lowest was 3-Imino-3h,5h- [1,2,4] Thiadiazolo [3,4-1] Isoindole (1,70 %). Eicosane, Docosane and Nonadecane present in average ammount (2-3%).

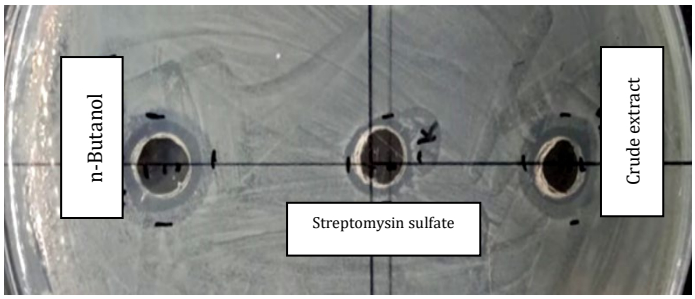

Figure 1. Antibacterial activity of *Streptomyces* sp. MB 106 crude extract

Table 1. Antibacterial activity against *Escherichia coli* ATCC 8739

Tested solution	Plate Number	Zone of inhibition (mm)
Crude extract	One	13
	Two	11.5
	Three	11.5
Mean		12
Streptomycin sulfate	One	16
	Two	15.5
	Three	14.5
Mean		15.33
	One	15
n-Butanol	Two	13.5
	Three	12.5
Mean		13.67

Table 2. Bioactive Compound detected in n-Butanol extract of *Streptomyces* sp. MB106

Bioactive Compound	Retention Time	Composition (%)
3-Imino-3h,5h- [1,2,4] Thiadiazolo [3,4-1] Isoindole	6.414	1,70
Naphtalene	8.462	43,89
Butane, 1,1-Dibutoxy	8.855	4,14
Butane, 1,1-Dibutoxy	10.186	18,99
Butyl (Tetradec-6-Yl) Sulfonate	29.375	1,73
Heneicosane	31.044	22,51
Eicosane	31.568	3,05
Docosane	31.740	2,67
Nonadecane	32	2,48

Discussion

Streptomyces sp. MB106 was isolated from the rhizosphere of the succulent plant *Euphorbia* sp. Its antibacterial activity towards *Escherichia coli* was tested. Antibacterial test against *Escherichia coli* ATCC 8739 showed a wide range inhibition zone varied from 11 to 13 mm with an average of 12 mm. The highest inhibition zone was 13 mm, it's 2.5

mm different from Streptomycin sulfate and 0.5 mm different from n-butanol. Although inhibition zone diameter still below the control our data suggest a potent antibacterial drugs candidate.

Streptomyces sp. crude extract was reported to have antibacterial antagonist activity on *Escherichia coli* and *Staphylococcus aureus* (Khattab et al., 2016). Other research on *Streptomyces olivaceus* LEP7 crude extract also reported inhibition zone to *Escherichia coli, Pseudomonas aeruginosa, S. aureus, Klebsiella* sp*., Acinetobacter* sp., and *Candida* sp. (Rajaram et al., 2020).

Gass chromatography-mass spectrometry (GC-MS) is one reliable method to identify volatile components. There are nine major bioactive compounds with various retention times through this technique. These bioactive compounds are suggested to have antibacterial properties.

Six out of nine bioactive compounds were reported previously to have antimicrobial activities. These compounds are butane, 1,1-dibutoxy, naphthalene, nonadecane, docosane, heneicosane and eicosane. Butane, 1,1-Dibutoxy has been reported to have potent antagonist activity against *Vibrio anguillarum* (Kawuri & Darmayasa, 2019). Nonadecane was reported to have antimicrobial activity, anti-HIV, and antioxidants (Kumar et al., 2018). Docosane had displayed antimicrobial activities against *Bacillus thuringiensis, Staphylococcus aureus, Escherichia coli, and Candida albicans* (Lammers et al., 2021)

Heneicosane has been reported to have antimicrobial effect on pathogenic microorganisms. Heneicosane extracted from *Plumbago zeylanica* was used for measuring the antimicrobial activity. heneicosane exhibited excellent antimicrobial activity against *Streptococcus pneumonia* with inhibition zone 31 ± 0.64 mm. It was also tested against *Aspergillus fumigatus* and showed inhibition zone 29 ± 0.86 mm at 10 µg/ml concentration (Vanitha et al., 2020).

Eicosane also reported having antimicrobial activities. Crude extract from *Streptomyces anulatus* NEAE-94 exhibit inhibition zone against *Staphylococcus aureus* NRRL B-313, multidrug-resistant *S. aureus* and *Bacillus subtilis* NRRL B-543 (El-Naggar et al., 2017). Antifungal activities of eicosane was effective againsti *Rhizoctonia solani* AG-3 strain KX852461 (Ahsan et al., 2017). Docosane, n-heneicosane, and nonadecane were twenty-two antibiotics bioactive compounds isolated from *Streptomyces anulatus* NEAE-94. (El-Naggar et al., 2017)

These six bioactive compounds need to be further analyzed for their potent, other antifungal, anticancer, and other beneficial properties. Therefore we suggest bioinformatics as in silico approach for future research.

Conclusion

We manage to obtain bioactive secondary metabolites of *Streptomyces* sp. MB 106. Crude extract showed an average of 12 mm inhibition zone against *Escherichia coli* ATCC 8739. Although inhibition zone diameter is still below the control, our data suggest a potent antibacterial drug candidate. There are nine bioactive compounds from *Streptomyces* sp. MB 106. Six of them have been reported to have potent antimicrobial activities. These compounds could be developed for future drugs candidate.

Acknowledgments

This research was supported and financed by Deputy for Strengthening Research and Development Ministry of Research and Technology/ National Research and Innovation Agency Under the Research Contract for the 2020 Fiscal Year Number: 064/E4.1/AK.04.PT/2021 (Hibah DIKTI Penelitian Dosen Pemula).

Declaration statement

The authors reported no potential conflict of interest

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