Turnitin-Antibacterial potential of Actinomycetes isolated from mangrove sediment in Tanjung Api-Api

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Short Communication: Antibacterial potential of Actinomycetes isolated from mangrove sediment in Tanjung Api-Api, South Sumatra, Indonesia

ROZIRWAN♥, HEBBRI ISKANDAR MUDA, TENGKU ZIA ULQODRY
Department of Marine Science, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Jl. Palembang-Prabumulih Km. 32, Indralaya, Ogan Ilir 30128, South Sumatra, Indonesia. Tel./fax.: +62-711-663375, ♥email: rozirwan@unsri.ac.id

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Abstract. *Rozirwan, Muda HI, Ulqodry TZ. 2020. Short Communication: Antibacterial potential of Actinomycetes isolated from mangrove sediment in Tanjung Api-Api, South Sumatra, Indonesia. Biodiversitas 21: 5723-5728.* Actinomycetes play an important role in the decomposition process of organic matter in mangrove sediments. This study was carried out to identify and evaluate the antagonistic activity of actinomycetes from mangrove sediment against *Escherichia coli* and *Staphylococcus aureus*. Identification of actinomycetes was performed based on morphological dan physiological characters. The antagonistic test was carried out against *Escherichia coli* and *Staphylococcus aureus*. A total of ten isolates had been successfully isolated and grouped into eight genera, including; *Oerskovia* H1, *Micrococcus* H2, *Nocardia* H3, *Sporichtya* H5, *Corynebacterium* H6, *Jonesia* H7, *Actinomyces* H10, and *Streptomyces* H8, H11, H12. Actinomycetes from mangrove sediment were gram-positive bacillus. The shape of the colony varied from circular to filamentous and irregular, with medium and large colony sizes. The colony appears white or yellow. The surface is convex and flat, aerobes and facultative anaerobes; capable of fermenting glucose. Five isolates have very strong growth inhibition activity against *E. coli: Nocardia* H3 (27.81mm ± 1.39), *Actinomycetes* H10 (27.75mm ± 2.48), *Corynebacterium* H6 (27.69mm ± 2.39), *Micrococcus* H2 (18.55mm ± 2.44), and *Streptomyces* H8 (17.92mm ± 2.70) while *Streptomyces* H8 (24.83mm ± 2.08), and *Actinomycetes* H10 (16.06mm ± 2.49) were active against *S. aureus*.

Keywords: Actinomycetes, antagonist test, *Escherichia coli*, mangrove sediment, *Staphylococcus aureus*

INTRODUCTION

Actinomycetes bacteria are grouped into gram-positive bacteria capable of producing various bioactive compounds (Kumar et al. 2019; Mahapatra et al. 2019; Kavitha and Vimala 2020; Shamikh et al. 2020). Actinomycetes are potential as antioxidant, antibacterial, antifungal, antifouling, antiviral (Chandra et al. 2020; Elkhateeb et al. 2020; Gacem et al. 2020; Gong et al. 2020; Hamed et al. 2020; Hassan et al. 2018; Kumar et al. 2020; Matthew et al. 2020; Pereira et al. 2020; Shaala et al. 2020; Yi et al. 2020). Therefore, the potential of marine natural products from actinomycetes collected from mangrove sediments needs to be explored.

Actinomycetes bacteria are found in muddy substrates, especially in mangrove areas. This area contains high nutrients, which come from the mainland through rivers and rainwater runoff. Organic materials from the land will be deposited in the mangrove area to form a thick mud. Mangrove ecosystems contribute very large amounts of nutrients to mangrove sediments (Reef et al. 2010). Mangrove sediments have an abundance of bacteria for the decomposition process that results in increasing soil fertility. It is also reported that actinomycetes can degrade waste (Waithaka et al. 2019).

Actinomycetes are the most widely distributed in mangrove sediments (Suresh et al. 2020). Actinomycetes exhibit antagonistic properties (Tistechok et al. 2019; Dede et al. 2020; Talpur et al. 2020). They can survive in a dynamic mangrove environment, such as changes in salinity, oxygen, temperature, pH, etc. Mangrove areas are greatly influenced by tides, salinity, pH, currents, temperature, and dissolved oxygen (Imamsyah et al. 2020),

The antagonist test was chosen because it is the most effective method for screening the potency of actinomycetes. It is a fast, easy method with tangible results. This test was conducted to determine the inhibitory activity of actinomycetes against pathogenic bacteria, namely *Escherichia coli* and *Staphylococcus aureus*. This study aims to isolate, identify, and determine the antibacterial potential of actinomycetes from mangrove sediment.

MATERIALS AND METHODS

Study area

Samples of mangrove sediment were taken in December 2018, located in the Tanjung Api-Api mangrove area (Latitude 2º22'17.05'' S, Longitude 104º48'19.74'' E) in South Sumatra, Indonesia. It is one of the most important coastal mangroves within the South Sumatra Province. Since 2004, this area has been used as a domestic port with busy shipping activities.

Sampling and Actinomycetes isolation

Sediment samples were taken from the mangrove sediment around the root of *Avicennia marina* using a pipe with a diameter of four inches at a depth of 0 to 25 cm. About 500 g of the samples were collected and placed in a plastic bag and then stored in a cool box. Actinomycetes from mangrove sediment were isolated based on the method by Dede et al. (2020) and Kavitha and Vimala (2020). Samples were serially diluted. *Actinomycetes* isolates were cultured on Starch Casein Agar (SCA) medium with a composition of 10 g of soluble starch, 4 g of yeast extract, and 16 g of agar per 1 L of dH2O. Samples were incubated at 28°C for seven days.

Morphological and physiological characterization

Morphological characters of actinomycetes were observed using a microscope. It includes colony characteristics, hyphal type, and vegetative hyphal growth. Physiological characterization was carried out using several biochemical tests. It includes catalase test, motility test, indole production test, carbohydrate fermentation, TSIA test, Simmons citrate test, Methyl Red test, Voges-Proskauer test, MIO test, LIA test, urease and gelatin test. Identification of actinomycetes refers to Bergey's Manual Determinative of Bacteriology (Goodfellow et al. 2012)

Antagonists test of actinomycetes isolates against pathogenic bacteria

The antibacterial potential of actinomycetes was carried out by an antagonistic test against *Escherichia coli* and *Staphylococcus aureus*. This method referred to (He et al. 2020; Loqman et al. 2009; Patil et al. 2001; Remya et al. 2008; You et al. 2005). The inhibition zone was measured twice at 24 h and 48 h, and the average inhibition zone was estimated based on the minimum diameter and added to the maximum diameter.

RESULTS AND DISCUSSION

Morphological and physiological characteristics of Actinomycetes

There were ten isolates of Actinomycetes bacteria successfully isolated from mangrove sediments in Tanjung Api-Api water (Figure 1). They are grouped into eight bacterial genera based on their macroscopic, microscopic, and biochemical characteristics. There are three isolates (H8, H11, and H12) identified as the genus *Streptomyces*.

Morphologically, the ten isolates had different characteristics in shape, size, elevation, color, surface, and margin. The shapes of isolates are generally circular and irregular; only one is filamentous. The sizes of isolates are medium and large, with convex and flat elevations. The colors of the cells are white and yellow, with uneven and evenly margins. All isolates are motile gram-positive, aerobics, and facultative anaerobes. Physiologically, the isolates showed generally positive reactions to sugars (Table 1).

Eight genera of actinomycetes from mangrove sediment samples are as follows: *Oerskovia* H1, *Microccus* H2, *Nocardia* H3, *Sporichthya* H5, *Corynebacterium* H6, *Jonesia* H7, *Actinomyces* H10, and *Streptomyces* H8, H11, H12. The genus *Streptomyces* was identified to be more dominant than others. However, the morphological and physiological characteristics of the three isolates differed slightly. The cell surfaces of H8 and H11 were characterized by a smooth, while H12 was rough. The cell margin of H8 was even, while H11 and H12 were uneven. The cell color of H8 and H12 was white, while that of H11 was yellow. Several differences were also found in the biochemical characteristics of the three isolates. It indicates that the three *Streptomyces* isolates are thought to be different species.

Antibacterial potential of actinomycetes isolates

The inhibition zone of actinomycetes isolates collected from mangrove sediments had high variation depending on the bacteria tested and the time of measurement (24 h and 48 h). The number of isolates that inhibited the growth of *Escherichia coli* was greater than *Staphylococcus aureus* (Table 2).

Based on the inhibition zone (Table 2), there are five isolates have very strong and strong inhibitory activity against *E. coli,* i.e., Genus *Nocardia* H3 (27.81 mm ± 1.39), *Actinomycetes* H10 (27.75 mm ± 2.48), *Corynebacterium* H6 (27.69 mm ± 2.39), *Micrococcus* H2 (18.55 mm ± 2.44), and *Streptomyces* H8 (17.92 mm ± 2.70). Two isolates had potent inhibition against *S. aureus*, i.e., Genus *Streptomyces* H8 (24.83 mm ± 2.08) and *Actinomycetes* H10 (16.06 mm ± 2.49).

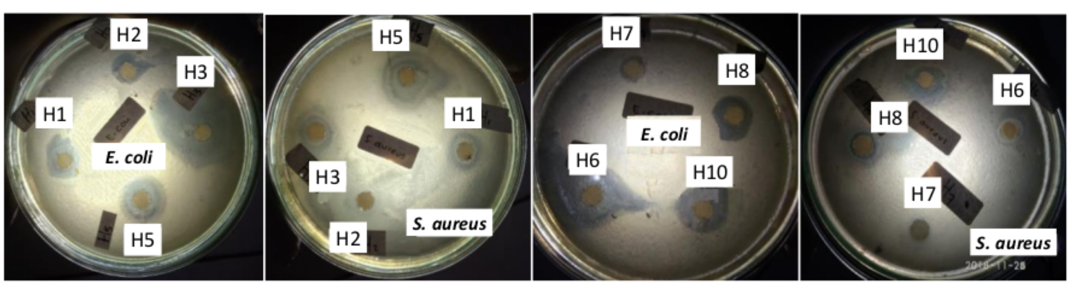

Figure 1. Inhibition zone of actinomycetes isolates against *Escherichia coli* and *Staphylococcus aureus*

Table 1. Characteristics of Actinomycetes isolated from mangrove sediment

Characteristics	Isolate code									
	H1	**H2**	**H3**	**H5**	**H6**	**H7**	**H8**	**H10**	**H11**	**H12**
Shape	Filamentous	Circular	Irregular	Circular	Circular	Irregular	Filamentous	Circular	Filamentous	Filamentous
Size	Large	Medium	Large	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Elevation	Flat	Convex	Flat	Convex	Convex	Flat	Convex	Convex	Convex	Convex
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Rough
Margene	Uneven	Evenly	Uneven	Evenly	Evenly	Uneven	Evenly	Uneven	Uneven	Uneven
Color	White	Yellow	White	Yellow	Cream yellow	White	White	Cream yellow	Yellow	White
Cell shape	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Motility	-	+	+	+	-	+	-	+	-	-
Gram	+	+	+	+	+	+	+	+	+	+
Oxygen	Facultative anaerobic	Facultative anaerobic	Aerobe	Facultative anaerobic	Aerobe	Facultative anaerobic	Aerobe	Facultative anaerobic	Aerobe	Aerobe
Gelatin	-	-	+	-	-	-	+	-	+	+
Indole	-	-	-	-	-	-	+	-	+	+
Urea	-	-	-	-	+	+	+	+	+	+
Citrate	-	-	-	-	-	+	+	-	+	+
Lysine iron agar	+	+	-	+	+	+	-	+	-	+
Mio	-	-	-	-	-	-	-	-	-	-
NaCl 4%	+	+	+	+	+	+	+	+	+	+
Methyl red	-	-	-	-	-	-	-	-	-	-
Voges Proskauer	+	+	+	-	-	-	-	+	-	+
TSIA	Acid	Catalyst	Acid	Acid	Acid	Catalyst	Catalyst	Catalyst	Catalyst	Catalyst
Catalase	+	+	+	-	+	+	+	+	+	+
Oxidase	+	+	+	-	-	-	+	+	+	+
Glucose	+	+	+	+	-	+	+	+	+	+
Sucrose	+	+	+	+	-	+	+	+	+	-
Lactose	-	-	-	-	-	+	-	-	-	+
Esculin	+	-	+	-	-	-	+	+	+	-
Arabinose	-	-	+	-	-	+	-	+	+	+
Cellobiose	+	-	+	-	-	-	+	+	+	+
Galactose	-	+	-	-	-	+	-	-	+	+
Fructose	+	+	+	-	-	+	+	+	+	+
Maltose	-	+	-	+	-	+	+	+	+	+
Melibiose	-	+	-	+	-	+	-	+	+	+
Sales	+	-	+	-	-	-	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	+
Mannitol	+	+	+	+	-	+	+	+	+	+
Xylose	-	+	-	+	-	+	-	+	-	-
Trehalose	-	+	-	+	-	+	+	+	+	+
Raffinose	-	-	-	-	-	+	-	+	+	-
Rhamnose	-	-	-	-	-	-	-	-	+	+
Genus	*Oreskovic*	*Micrococcus*	*Nocardia*	*Sporichthya*	*Corynebacterium*	*Jonesia*	*Streptomyces*	*Actinomyces*	*Streptomyces*	*Streptomyces*

Table 2. The inhibition zone of actinomycetes against *Escherichia coli* and *Staphylococcus aureus*

Genus	Isolates code	Inhibition zone (mm)			
		E. coli		***S. aureus***	
		24 hour	**48 hour**	**24 hour**	**48 hour**
Oerskovia	H1	14.31±2.21	14.52±2.33	9.28±2.09	9.38±2.12
Micrococcus	H2	18.45±2.51	18.55±2.44	9.35±2.30	9.58±2.40
Nocardia	H3	27.70±1.34	27.81±1.39	14.38±2.80	14.41±2.81
Sporichthya	H5	14.13±2.61	14.30±2.40	12.08±2.48	12.18±2.47
Corynebacterium	H6	27.68±2.37	27.69±2.39	9.48±0.32	9.57±0.34
Jones	H7	6.71±0.02	6.81±0.05	5.80±0.04	6.39±0.05
Streptomyces	H8	17.70±2.72	17.92±2.70	24.49±1.71	24.83±2.08
Actinomycetes	H10	27.73±2.51	27.75±2.48	15.99±2.53	16.06±2.49
Streptomyces	H11	5.78±1.34	5.89±1.43	4.96±0.43	4.96±0.41
Streptomyces	H12	4.63±0.99	4.66+0.97	-	-

Discussion

Ten isolates were successfully isolated from mangrove sediments in Tanjung Api-Api that are grouped into 8 genera. These genera are widely reported in sediments (Al-Dhabi et al. 2020; Arifiyanto et al. 2020; Bolorunduro et al. 2019; Elhagrassy 2018; Shah et al. 2017; Abidin et al. 2016). The genus *Actinomyces* is also reported to be gram-positive bacteria and facultatively anaerobic. It can also ferment sugars, catalase, and Voges-Proskauer positive (Goodfellow et al. 2012).

The genus *Corynebacterium* was also found from sea sediments in Thailand (Leetanasaksakul and Thamchaipenet 2018), the sediment from Valparaíso bay, Chile (Claverías et al. 2019). *Corynebacterium* is gram-positive bacilli and produces catalase (Goodfellow et al. 2012). The genus *Jonesia* is motile, gram-positive bacilli, catalase-positive with oxidase-negative, does not hydrolyze indole, hydrolyzes urea, and citrate, liquefies, and ferments sugars such as glucose, lactose, and saccharose (Goodfellow et al. 2012). The genus *Micrococcus* was found in the Nellore coastal regions of Bapatla and Vishakhapatnam, South India (Kumari et al. 2020). Generally, bacterial isolates in the environment are halophilic and have the potential to be applied in industry.

Nocardia is found in tropical mangrove sediments in Malaysia (Lee et al. 2014). This species was found by (Fahmy 2020). (Goodfellow et al. 2012) It has white, orange, pink, and red colony colors, irregular colony forms, gram-positive, aerobic, rod-shaped, motile and non-motile, catalase-positive, and can hydrolyze several types of sugars. It is halophilic and can remodel cellobiose, dextrins, cellulose, and agar with palmitate and stearate, substrates, that are commonly used to degrade cellobiose. (Person et al. 1991). *Oerskovia* was found by (Dimri et al. 2020). *Sporichthya* was also found in sediments, a facultatively anaerobic gram-positive bacterium. It is a chemoorganotropic and mesophilic, motile, and sensitive to high temperatures (Ahmad et al. 2019).

The inhibitory zones produced by actinomycetes isolates against pathogenic bacteria were varied. Five isolates showed a strong inhibitory zone, namely, *Nocardia* (H3), *Actinomycetes* (H10), *Corynebacterium* (H6), *Micrococcus* (H2), and *Streptomyces* (H8) against *E.coli*. The inhibitory zone is presumably related to the production of active antibacterial compounds by these isolates (Ahmad et al. 2019; Al-Farraj et al. 2020; Fahmy 2020; Sapkota et al. 2020). Several previous studies showed that actinomycetes have good antibacterial activity. For example, *Nocardia* from Indian mangrove ecosystems (Manikkam et al. 2019), *Actinomyces* from Nipah mangrove sediment (Yanti et al. 2020), *Corynebacterium* the sediment of Valparaíso bay, Chile (Claverías et al. 2019), *Micrococcus* from seawater (Kumari et al. 2020), and *Streptomyces* from mangrove soil sediment (Al-Dhabi et al. 2019; Al-Farraj et al. 2020; Fahmy 2020; Kripra et al. 2020; Sabido et al. 2020).

Five isolates showed more potent antibacterial activity against *E. coli* than *S. aureus*. They were more effective in inhibiting gram-negative bacteria than gram-positive bacteria. Gram-positive bacteria have a thicker cell membrane than gram-negative bacteria (Christofferson et al. 2020). Therefore, the active antibacterial compounds might be easier to penetrate the membrane cell of gram-negative bacteria. Antibacterial activity is also influenced by the method, type, and level of resistance of the tested microbes.

In conclusion, total of ten actinomycetes bacteria isolated from the mangrove sediments in Tanjung Api-Api, South Sumatra, and was grouped into eight genera, i.e., *Oerskovia* H1, *Micrococcus* H2, *Nocardia* H3, *Sporichtya* H5, *Corynebacterium* H6, *Jonesia* H7, *Actinomyces* H10, and *Streptomyces* H8, H11, H12. Five isolates showed very strong antibacterial activity; namely, *Micrococcus* H2, *Nocardia* H3, *Corynebacterium* H6, *Streptomyces* H8, and *Actinomycetes* H10 against gram-negative bacteria. Their antibacterial potential is more likely to inhibit gram-negative than gram-positive bacteria. Actinomycetes isolated from mangrove sediment have the potential as natural medicine.

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