Potential of Actinomycetes from Bora Hot Springs Central Sulawesi to Produce Antibacterial Compounds

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Abstract. Actinomycetes are Gram-positive bacteria that could produce antibacterial compounds, and it is able to survive in high temperatures environment such as hot springs. In this study, Actinomycetes were isolated from Bora hot springs before, characterized, and determined their potential in – other antibacterial activity. The isolation method was to pour the plate using SCA (Starch Casein Agar) media. The isolates were then purified by using the streak plate method and characterized their properties macroscopically and microscopically. Antibacterial activity was performed against *Escherichia coli* and *Staphylococcus aureus*. The results showed that there were 5 Actinomycetes isolates (namely AB-1, AB-2, AB-3, AB-4, AB-5) with different characters on each isolate. Isolates AB-1 and AB-3 were able to inhibit the growth of *E. coli* and *S. aureus*, while AB-2, AB-4, and AB-5 did not inhibit either *E. coli* or *S. aureus*. Based on the test results, it can be concluded that Actinomycetes isolates from Bora hot springs have potency in producing antibacterial compounds for commercial purposes.

Keywords: Bora Hot Spring · Actinomycetes · Antibacterial · *S. aureus* · *E. coli*

1 Introduction

Actinomycetes are Gram-positive filamentous bacteria. It has the potential to produce secondary metabolites with diverse characteristics and has colony pigmentation, mycelium, and pigments that aren't usually the same when grown on various media [1]. Actinomycetes are a group of microorganisms that live widely in nature, such as in hot springs [2].

There are several hot springs in Central Sulawesi, Bora hot spring is a hot spring located in Bora Village, Sigi Biromaru District, Sigi Regency which has temperatures ranging from 55–81 °C. Previous research has succeeded in isolating thermophilic bacteria from Bora hot springs. The bacteria isolated from Bora Hot Spring have the potential to produce amylase [3]. Thus, it showed that Bora hot spring has the potential as a natural habitat for Actinomycetes. Based on it, this study will evaluate whether the Bora hot spring contains Actinomycetes organisms that have the potential to produce antibacterial compounds.

N. I. Inda et al. (Eds.): ISST 2022, APR 7, pp. 221–227, 2023.
https://doi.org/10.2991/978-94-6463-228-6_25

Samples used in this study were obtained from Bora hot Springs Central Sulawesi which aimed to isolate Actinomycetes, characterize Actinomycetes isolates, and determine the Actinomycetes antibacterial activity isolates from Bora hot springs, Central Sulawesi. The outcome indicates that Actinomycetes isolates from Bora hot springs can be potentially produce antibacterial compounds.

2 Material and Method

2.1 Material

The samples taken from Bora hot springs, Central Sulawesi, starch, casein, KNO_3, MgSO4, K_2HPO_4, NaCl, $CaCO_3$, $FeSO_4$, Agar, chloramphenicol, tryptone, yeast extract, Nutrient Agar (NA) media, Gram staining reagents and test bacteria which are pathogenic (*Escherichia coli* and *Staphylococcus aureus*).

2.2 Methods

Methods: Water samples were taken randomly at Bora hot springs, Central Sulawesi, then isolated using the pour plate method on SCA (Starch Casein Agar) media with 55 °C incubation temperature. The isolates that isolated before, then purified to obtain pure isolates of Actinomycetes using streak plate method. Then, characterized macroscopically and microscopically. The isolates were then tested for their inhibition ability toward pathogenic bacteria growth through an antibacterial activity test using the Well Diffusion Agar Method.

3 Result and Discussion

The results of this study based on sample isolation from Bora hot spring Central Sulawesi, there were 10 Actinomycetes isolates. Then, each isolates were purified by using streak plate method and obtained 5 pure Actinomycetes isolates namely AB-1, AB-2, AB-3, AB-4, and AB-5. Fifth isolates were characterized macroscopically and microscopically. Macroscopic characterization was carried out by observing Actinomycetes colonies visible on the media on petri dish. Based on the observations, it appears that fifth isolates have different characters but there are also some characters that shows similar. This result in-line with previous research by Sulistyanto & Trimulyono [4]. The differences that occur in each character of Actinomycetes isolates are caused by the different biological role of each isolate [5]. Macroscopic observations data are presented in Fig. 1 and Table 1.

Colony morphology observation through the observations according to the procedure that had been reported by Suloi *et al.,* there were isolates that had white aerial mycelium, brownish white, and brown substrate mycelium. This is in-line with isolates AB-2, AB-3, AB-4 and AB-5 [5]. Another similarity with previous research are isolates AB-1 and AB-4 with convex elevation, isolates AB-2 and AB-5 with white colonies. This is also in-line with the study from Sulistyanto & Trimulyono which had isolates with convex elevation characteristics and white colonies [4].

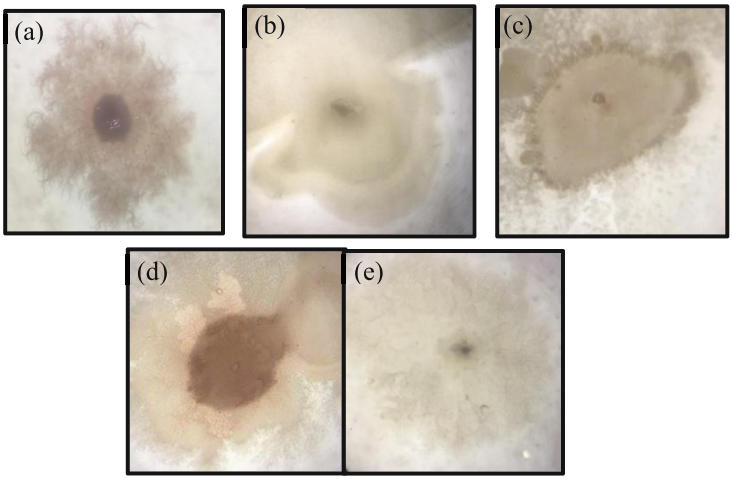

Fig. 1. Macroscopic observation after being incubated for three days at 55 °C. Each isolate has different characteristic. (a) Isolate AB-1 (b) Isolate AB-2 (c) Isolate AB-3 (d) Isolate AB-4 (e) Isolate AB-5.

Table 1. Macroscopic observations of Actinomycetes isolates

No.	Isolate	Pigmentation	Shape	Elevation	Edge	Aerial mycelium	Substrate mycelium
1	AB-1	Reddish white	Rhizoid	Convex	Rhizoid	Reddish white	Reddish white
2	AB-2	Pale white	Irregular	Flat	Irregular	Pale white	Yellowish white
3	AB-3	Brownish white	Irregular	Flat	Irregular	Brownish white	Brownish white
4	AB-4	Brownish red	Irregular	Convex	Irregular	Brownish red	Light brown
5	AB-5	Pale white	Filamentous	Flat	Filamentous	White	White

In microscopic observations carried out by Gram staining, fifth isolates had coccus cell shape and belongs to Gram positive (Fig. 2 and Table 2). This is in-line with the study from Sulistyanto & Trimulyono which is all of their Actinomycetes isolates obtained were Gram positive [4]. Fifth isolates had various sizes ranging from 1.20 μm to 1.59 μm. Actinomycetes cell size characteristic is 1–2 μm [6].

Based on macroscopic and microscopic data, it was shown that each isolate had similarities in characters as well as differences. Similarities were found in the type of Gram and the cell shape of the fifth isolates, namely Gram positive and coccus-shaped. Meanwhile, the differences in character were found in the morphology of fifth isolates and their cell diameters ranging from 1.20 to 1.59 μm. This indicates that the isolates

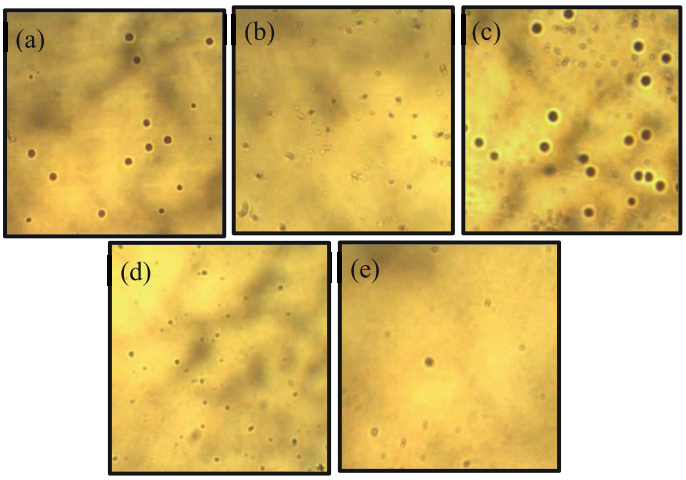

Fig. 2. Microscopic observation carried out by Gram staining. Each isolate has coccus cell shape and Gram positive bacteria. (a) Isolate AB-1 (b) Isolate AB-2 (c) Isolate AB-3 (d) Isolate AB-4 (e) Isolate AB-5

Table 2. Microscopic observations of Actinomycetes isolates

No.	Isolate	Cell shape	Gram	Vegetative cell size p x l (μm)
1	AB-1	*Coccus*	+	1,46
2	AB-2	*Coccus*	+	1,20
3	AB-3	*Coccus*	+	1,59
4	AB-4	*Coccus*	+	1,57
5	AB-5	*Coccus*	+	1,44

obtained belong to Actinomycetes organism and indicate that each isolate is a different species.

Fifth isolates were then tested for their ability to produce antibacterial compounds using pathogenic bacteria, namely *E. coli* which represented Gram negative bacteria and *S. aureus* which represented Gram positive bacteria. *E. coli* and *S. aureus* are bacteria that are resistant to several antibiotics. *E. coli* are resistant to antibiotics such as penicillin and amoxicillin, while *S. aureus* are resistant to the antibiotic fosfomycin [7, 8].

The antibacterial activity test used well diffusion agar method on NA media using 2% chloramphenicol (positive control) and aquadest (negative control). According to Ramadhani & Sulistyani (2018), chloramphenicol was used as a positive control to determine the comparative value of the antibacterial potential between Actinomycetes and chloramphenicol [9]. This is because chloramphenicol is a broad-spectrum antibiotic [10]. The results of the antibacterial activity can be seen in Fig. 3.

The positive control using 2% chloramphenicol showed the largest inhibition zones with diameters of *S. aureus* and *E. coli* were 21.73 mm and 17.39 mm, respectively.

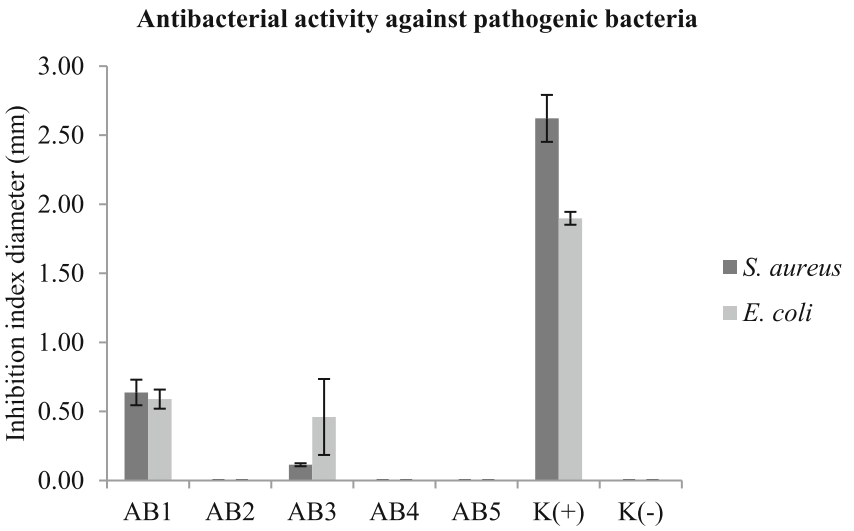

Fig. 3. Antibacterial activity of fifth Actinomycetes isolates (AB-1, AB-2, AB-3, AB-4, and AB-5) from Bora hot springs against test bacteria S. aureus (Gram positive) and E. coli (Gram negative). Error standard based on the average diameter of inhibition index. Inhibition was measured based on the diameter of the clear zone formed around the well after being incubated for 24 h at 37 ºC.

This is because chloramphenicol is a broad-spectrum antibiotic, so it can inhibit the growth of Gram-positive and Gram-negative bacteria. While the smallest inhibition zone was found in the negative control using aquadest with 0 mm diameter for each pathogenic bacterium. This is because in aquadest there are no compounds that can inhibit the growth of pathogenic bacteria.

Based on these data, it can be seen that among the five isolates there were only 2 isolates that were able to inhibit the growth of pathogenic bacteria, namely isolates AB-1 and AB-3. Isolate AB-1 inhibited *S. aureus* with 9.83 mm diameter and 9.54 mm in *E. coli*. Meanwhile, isolate AB-3 inhibited *S. aureus* with 6.69 mm diameter and 6.37 mm in *E. coli*. Compared with chloramphenicol (positive control), it was seen that chloramphenicol showed the greatest inhibition compared to isolates AB-1 and AB-3. This is due to the difference in the rate of diffusion in the agar medium.

According to Kumala *et al.* (2015) an indication that there is antibacterial activity of Actinomycetes isolates is evidenced by the presence of a clear zone around the well, the presence of a clear zone also indicates that Actinomycetes isolates can inhibit the growth of pathogenic bacteria [11]. Based on the data above, it can be seen that the inhibition zone produced after being incubated for 24 h at 37 °C, there were only 2 isolates (AB-1 and AB-3) which showed antibacterial activity and were able to inhibit both pathogenic bacteria. The inhibition zone is formed due to the presence of antibacterial compounds resulting from secondary metabolites which are secreted into media that can inhibit growth and kill pathogenic bacteria [5].

The resulting inhibition zone indicated that isolates AB-1 and AB-3 were able to produce antibacterial compounds that could inhibit pathogenic bacteria. This is because

Actinomycetes secondary metabolites contain flavonoids and alkaloids. According to previous research from Guntur through the results of phytochemical screening using the TLC method [12]. Flavonoid compounds denature microorganism cell proteins and damage cell membranes while alkaloid compounds interfere with peptidoglycan constituent components so that the cell wall layer is not formed intact. The secondary metabolites produced by Actinomycetes show large numbers with diverse biological effects, such us antimicrobial activity [13].

4 Conclusion

Actinomycetes from Bora hot springs Central Sulawesi (AB-1, AB-2, AB-3, AB-4, and AB-5) belong to the Gram positive group with coccus cell shape. Fifth isolates had different characters, indicating that each isolates were different species. Isolates AB-1 and AB-3 that had been obtained have the potential to produce antibacterial compounds that can be developed commercially.

Acknowledgement. Thank you to the Bora hot springs management for giving access to the location. Thank you to Ms. Sami Bukang, S.P., Rian Aristiawan, Dewi Purnamasari, Nurul Rahma, and Titik Nur Vitasari who had helped a lot during in the laboratory works.

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