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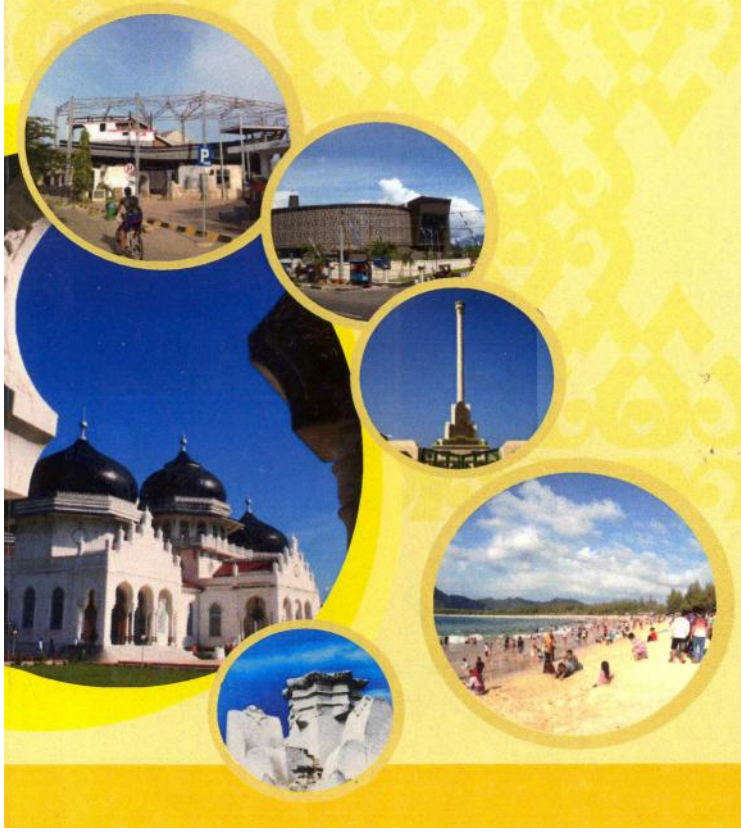

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DARUSSALAM, BANDA ACEH

Antibiotic Production From Thermophilic Jaboi Sabang Local Isolate : Using TSB Medium

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Abstract

The goal of this study was to isolate antibiotic produced using TSB medium from thermophilic Jaboi Sabang local isolate. Jaboi Sabang isolate exhibit antibiotic activity based on the formation of clear hallo around the colonies observed by well difusion methods using *Escherichia coli* and *Staphylococcus aureus* as indicator. The clear hallo intensity of the isolates showed high intensity to antibiotic production. The Jaboi Sabang isolate showed highest antibiotic activity when incubated at incubation time of 112 hours. In conclution, we assumed that *Jaboi Sabang* isolate produced antibiotics.

Keywords : Antibiotic, Jaboi Sabang Isolate, Tryptone Soy Broth (TSB)

Introduction

Antibiotics was a compound that have the effect of suppressing or killing a biochemical process in microorganisms (Usta and Demirkan 2013; Brock, 2009). Antibiotics compound can be function in their toxicity as bacteriostatic, bacteriocidal and bacteriolisis (Brock, 2009). Antibiotic drugs have been used for therapeutic in human, veterinary and agricultural purposes (Gebreyohannes, *et al*., 2013). Antibiotic compound can be produced of gram-positive and negative bacteria, fungi, plant, Actinomycetes and algae (Gesheva 2010; Brock, 2009, Gebreyohannes, *et al*., 2013). *Bacillus* species was one of the largest antibiotic producer from microorganism. The *Bacillus* genus have been studied 167 antibiotics produced, including 66 derived (Usta and Demirkan 2013).

The genus *Bacillus* has been studied and report from the ability of produce and secrete large of antibiotics examples bacitrasin, polymixin, colistin, gramicidin, tyrothricin cerexin, zwittermicin circuli, difficidin, subtilin, mycobacillin (Usta and Demirkan 2013; Brock, 2009). This antibiotics have a high economic value because it can be used as a topical antibiotic, inflammation, and used as a growth promoter. Several research reports that in genus *Bacillus* produces the polypeptide antibiotics. The antibiotic polypeptide has molecular weight between 270 to 4500 Da (Crueger and Crueger, 1989).

Indonesia is one of the most tectonically active area in the world with a number of volcanoes and a lot of geothermal area as highly potential as habitat of thermophile and hyperthermophile microorganism. Some research findings shown diversity of isolated thermophilic microorganisme have been known to produce antibiotic compound (Kulkarni M.S. and Kanekar P.P., 2011; Venugopalan *et al.*, 2008; Fandi *et al.*, 2012). Thermophiles microorganisme have been know as source of new antibiotic molecules (Venugopalan *et al.*, 2008).

Antibiotic production can be influenced by genetic of producing strain and different environmental conditions such as the incubation time, temperature, pH and nutrients of medium. This condition can be modified to expand the range of the antbiotic produced (Usta and Demirkan 2013; Furtado *et al.*, 2005). Therefore, the purpose of this study was to isolate antibiotic using TSB medium by Jaboi *Sabang* local as *Bacillus genus* in Aceh hot springs, Indonesia.

Material and Methods

Growth Curve

An extracellular antibiotics from Jaboi Sabang isolate was produced on the media TSB-consisting of 1,709 (b/v) casein, 0,309 (b/v) *Soybean meal*, 0,25 % (b/v) dextrose, 0,509 % (b/v) NaCl and 0,25 % (b/v) K_2HPO_4 and 2.5 % trypton soy broth with the fermentation time (0-7 days). Antibiotict was produced at 70 °C, pH 7, shaker incubator, 150 rpm. The supernatant of the culture after centrifugation (10000 g, 10 min) at 4 °C was used to determine antibiotics activity from crude extracts.

Antibiotic Assay

Antibiotic activity of the crude axtract was carried out by well difusion method as described by Kirby Bouer. Cell free supernatans from the stationary phase was applied as 100 uL drops in 6 mm diameter well of petri plates containing test microorganisme (*Escherichia coli* and *Staphylococcus aureus*) on Mueller Hinton Agar (MHA) medium. The extracts antibiotic were applied on the MHA and left at room temperature for 15 minute to allow the ectract to diffuse. The plates were incubated at 37 °C for 18-24 hour. The inhibition zone were measure after 24 jam incubation time.

Results and Discussion

Growth Curve Using TSB Medium

The growth curve was quite important in understanding a pattern of antibiotics production. Growth curve of Jaboi Sabang isolate was using TSB medium. The Jaboi Sabang isolate grew optimally at pH 7.0 and 70 °C. The Jaboi Sabang isolate showed early stationary phase at 96-120 hour. Antibiotict can be produced at stasionary phase. This phase could be

determined to antibiotics assay (Figure 1). The result indicates that Jaboi Sabang isolate showed excellent growth in TSB medium at 70 °C, pH 7.0. The result of this assay discovered that at 104-120 hours appeared the early and end of stationary phase. This result suggest that the crude extract at stationary phase can be assayed to antibiotict activity from Jaboi Sabang isolat

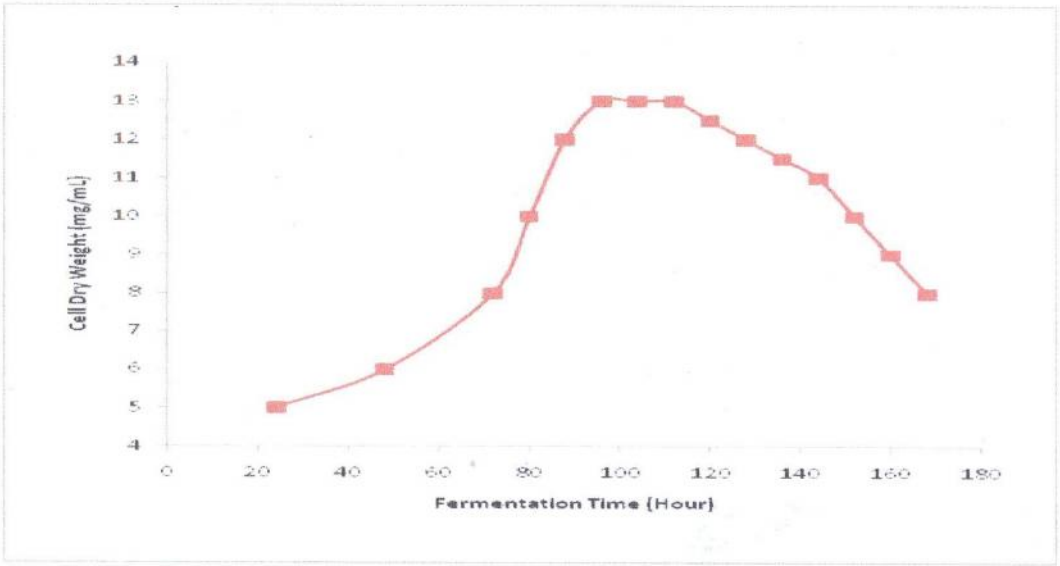

Figure 1. Growth Curve of Jaboi Sabang Isolate TSB Medium

Clear Inhibition Zone

Antibiotics activity was exhibited based on the formation of clear zones around the colonies observed by well difusion methods against Gram-negative bacterium (*Escherichia coli*) and Gram-positive bacterium (*Staphylococcus aureus*) as bioindicators. Crude extract showed antibiotic activity at stasionary phase. The highest antibiotic activity from Jaboi Sabang isolate indicated clear zone with 21, 18 mm at 112 hours of fermentation against *Escherichia coli* and *Staphylococcus aureus*, respectively (Table 1).

Table 1. Antibiotic activity of Jaboi Sabang Isolate

Fermentation Time (Hour)	Inhibition Zone in mm	
	Escherichia coli	*Staphylococcus aureus*
104	17	16
112	21	18
120	19	14

The antibiotic activity of crude extract from local isolate was dissimilar between gram negative and gram positive bacteria strains. The result clearly showed that a gram negative bacteria was succeptible to the assayed crude extract compared to gram positive. Based on clear zone showed antibiotic activity higher than to *Escherichia coli* from *Staphylococcus aureus*. Antibiotic activity showed from stasionary phase : 104, 112 and 120 incubation time. This data indicated that Jaboi sabang isolate have been started secreting secondary metabolites after 104 hours of incubation and increased to 112 hours of incubation. At 120 hours to antibiotic production decreased to *Escherichia coli* and *Staphylococcus aureus* as indicator. While on the fermentation time was no longer producing antibiotics. *Bacillus* antibiotics are generally produced at the early stages of the the stationary phase. Therefore, the relationship between antibiotic synthesis and growth of bacteria. At 112 hours of incubation, the decrease in growth of cell bacteria was paralleled by increase antibiotic activity (Figure 1 and Table 1). At 120 hours, the bacteria were in the end of stasionary phase or early the death phase. Antibiotic biosynthesis was usually produced at the beginning of the stationary and was optimum at middle of stationary phase. The result suggested that Jaboi Sabang isolate can be produced antibiotic activity at stasionary phase and optimum activity in middle of stationary phase. Therefore, Jaboi Sabang Isolate can be a potential source for antibiotic production, which leads to development of new drug for treatment of infectious diseases.

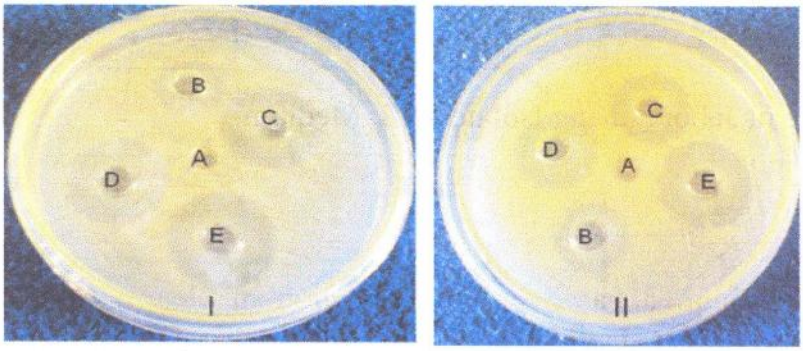

Figure 2. Antibiotic activity of Jaboi Sabang Isolate against *Escherichia coli* (I), *Staphylococcus aureus* (II) in the agar difusion method (A = Negative Control ,B = 120 hour fermentation , C and D = 104 hour fermentation , E = 120 hour fermentation (A = Negative Control ,B = 120 hour fermentation , C and D = 104 hour fermentation , E = 120 hour fermentation

Conclusions

The best finding of antibiotic activity showed highest activity of antibiotic when incubated at incubation time of 104 -120 hour with TSB medium.
The Jaboi Sabang was found to be antibiotic producer and their activities ranged from broad spectral (positive and negative gram inhibition).

Acknowledgements

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