



ECOPOTENTIAL *Bacillus* sp. FROM KRUENG ACEH: BIOREMEDIATION AND ANTIMICROBIAL ACTIVITY

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Received: 21 October 2024

Accepted: 28 December 2024

Published: 31 December 2024

ABSTRACT

Bacillus bacteria are bacteria that are tolerant to heavy metal toxicity and are able to reduce heavy metals in the environment by bioaccumulation or bioabsorption. This research aims to determine how resistant *Bacillus* sp. against the heavy metal Fe, the growth curve of *Bacillus* sp. affected by Fe metal, characteristics of the Krueng Aceh pathogenic bacteria and the antimicrobial activity of *Bacillus* sp. against pathogenic microbes. This research method is descriptive and experimental. Based on the research results, it is known that *Bacillus* sp is resistant to Fe metal at concentrations of 2000 ppm and 2500 ppm. Results of measuring the growth curve of *Bacillus* sp. exposed to Fe metal with a concentration of 3000 ppm entered the lag phase (adaptation) at hour 0. Then the log (exponential) phase starts from the 12th hour to the 30th hour, then the stationary phase starts from the 30th hour to the 42nd hour. The results of the test for the characteristics of pathogenic bacteria showed that there were 14 isolates of pathogenic bacteria, namely 4 genus *Klebsiella*, 4 genus *Enterobacter*, and 6 genus *Staphylococcus*. The results of the antimicrobial activity test for *Bacillus* sp., which was tested on pathogenic microbes, namely *Klebsiella* sp., and *Staphylococcus* sp., had a weak inhibitory category, namely 2.71, 0.51, this shows that *Bacillus* sp. unable to inhibit these pathogenic microbes.

Keywords : *Bacillus* sp, heavy, antimicrobial

ABSTRAK

Bakteri *Bacillus* merupakan bakteri yang toleran terhadap toksisitas logam berat serta mampu mereduksi logam berat di lingkungan dengan cara bioakumulasi atau bioabsorbs. Penelitian ini bertujuan untuk mengetahui bagaimana resistensi *Bacillus* sp. terhadap logam berat Fe, kurva pertumbuhan *Bacillus* sp. tercekam logam Fe, karakteristik bakteri patogen Krueng Aceh dan aktifitas antimikroba *Bacillus* sp. terhadap mikroba patogen. Metode penelitian ini ialah deskriptif dan eksperimental. Berdasarkan hasil penelitian diketahui bahwa *Bacillus* sp. resisten terhadap logam Fe dengan konsentrasi 2000 ppm dan 2500 ppm. Hasil pengukuran kurva pertumbuhan *Bacillus* sp. tercekam logam Fe memasuki fase lag (adaptasi) pada jam ke-0. Kemudian fase log (eksponensial) dimulai dari jam ke-12 sampai jam ke-30, selanjutnya fase stasioner dimulai dari jam ke-30 sampai dengan jam ke-42. Hasil uji karakteristik bakteri patogen didapatkan 14 isolat bakteri patogen, yaitu 4 genus *Klebsiella*, 4 genus *Enterobacter*, dan 6 genus *Staphylococcus*. Hasil uji aktifitas antimikroba *Bacillus* sp., yang diujikan pada mikroba patogen yaitu *Klebsiella* sp., dan *Staphylococcus* sp. memiliki kategori daya hambat lemah yaitu 2,71, 0,51 hal ini menunjukkan bahwa *Bacillus* sp. tidak mampu menghambat mikroba patogen tersebut.

Kata kunci : *Bacillus sp*, logam berat, Antimikrobia

Introduction

The city of Banda Aceh is an area traversed by rivers, one of which is Krueng Aceh. The river flows into two parts from the city center, namely the southern and northern parts, the Krueng Aceh river is also the longest and largest river. The development and growth of the City of Banda Aceh is inseparable and separate from the Krueng Aceh River. Krueng Aceh plays a strategic role as a supporter of urban activities and has enormous beauty in shaping the face of the city and improving the urban quality of Banda Aceh. The Krueng Aceh River Basin (DAS) has an area of $\pm 1,755 \text{ km}^2$ with a length of $\pm 145 \text{ km}$, with upstream flow in Cot Seukek, Aceh Besar to downstream at Lampulo Fisherman's Pier, Banda Aceh (Rahmat *et al.*, 2018).

Pollution can have a negative impact on the environment and waters, Muara Krueng Aceh is a source of pollution that is thought to have this impact. The waters of the Krueng Estuary are thought to have experienced pollution in the form of organic waste, domestic waste, shipping activities, heavy metals and oil spills which continue to increase over time and exceed the limit (Hadi *et al.*, 2018). Water pollution is the entry of living creatures, energy, substances and other components into the water or changes in the structure of the water due to natural processes or human activities so that the impact is that the quality of the water decreases and it can no longer be used according to its function (Pratiwi, 2020).

Heavy metals are natural parts found in the earth's crust and are not easily destroyed or degraded and heavy metals are dangerous substances because they can cause bioaccumulation. Bioaccumulation is an increase in the concentration of pollutants in an organism's body over a long period of time, compared to the concentration of pollutants in the environment (Adhani, 2017).

Iron (Fe) is a fundamental microelement for the body, iron is needed in the process of blood formation (hematopoiesis), namely in the synthesis of hemoglobin. Even though it is needed in the body, excessive levels can cause damage to the intestinal walls. Fe can also accumulate in the alveoli, resulting in lack of lung function (Murray, 2018). Based on the results of measuring the Fe concentration in the waters of Krueng Aceh, Jln. Teuku in Anjong, Gampong Jawa, Kec. Kuta Raja, Banda Aceh City, Aceh is 1886 mg/kg and shows that Fe metal in these waters is above the quality standard threshold. According to Republic of Indonesia Government Regulation No. 22 of 2021, the quality standards for Cd, Pb and Fe metals are 0.01 each; 0.03; and 0.3 mg/L (Kiswando *et al.*, 2022).

According to Rahadi *et al.*, (2019) *Bacillus* bacteria are bacteria that have tolerance to heavy metal toxicity and have the ability to eliminate heavy metals in the environment by absorbing them. The *Bacillus* genus is able to inhibit the growth of other microorganisms because *Bacillus* can produce antibacterial compounds, namely bacteriocins. Bacteriocins are peptide compounds that have the advantage that they can inhibit the growth of other microorganisms, are not toxic, and are easily degraded by proteolytic enzymes, and are not harmful to the microflora in the intestine because they are easily digested by digestive enzymes (Ambarwati & Ibrahim, 2021).

Methods

1. Rejuvenation of *Bacillus* sp.

Bacillus sp. bacterial isolate. is an ID bacterium isolated from Krueng Aceh and obtained from the Laboratory collection of the Multifunctional Building, Faculty of Science and Technology, Ar-Raniry State Islamic University, Banda Aceh. Bacterial isolates were taken by rejuvenating the bacteria, namely by taking 1 batch of bacterial isolates aseptically and growing them on Nutrient Agar (NA) medium, then incubating at a temperature of 37 °C (Rismawati, 2019).

2. Isolation and Identification of Test Microbes

River water sampling was carried out at the mouth of the Krueng Aceh river, Street of Teungku in Anjong, Gampong Jawa, District Kuta Raja, Banda Aceh City, Aceh Province. The sampling coordinates are Latitude 5.571031°N and Longitude 95.321576°E. The water samples that have been taken are isolated, identified macroscopically, then followed by microscopic identification, namely by gram staining. Next, biochemical testing is carried out including the TSIA test (Triple Sugar Iron Agar), Indole test, MRVP (Methyl red-Voges Proskauer) test, citrate test and catalase test.

3. Bacterial Resistance Test Against Fe Metal (Iron)

Resistance testing was carried out with 3 treatments (control, concentration 2000 ppm, 2500 ppm). It is carried out using the Kirby Bauer method, namely, a sterile cotton bud is placed in a test tube containing bacterial suspension, and then streaked onto a petri dish containing MHA media, then a paper disk is placed on top. Then paper disks that have been soaked in Fe with varying concentrations, namely: 2000 ppm and 2500 ppm are placed on MHA media that has been inoculated with *Bacillus*. Then incubated for 24 hours at 37 °C in an incubator. The formation of a clear zone in the disc area is a sign that bacteria have inhibitory abilities (Novaryatiin et al., 2018).

4. *Bacillus* Growth Curve Measurement

Bacterial growth curve measurements were made using Optical Density (OD) every 6 hours for 72 hours, namely by taking bacteria that were able to resist Fe with the fewest inhibition zones. This curve was measured using Nutrient Broth (NB) media, namely bacteria were inoculated into a tube containing 10 ml NB media and incubated for 24 hours. Next, aseptically, 10 ml of bacteria was inoculated into 90 ml of NB media with the addition of a Fe concentration of 3000 ppm and then shaker at a speed of 200 rpm. Every 6 hours, 1 ml of culture was taken and then put into a cuvette for measuring the OD value with a wavelength (λ) of 600 nm. OD measurements start from hour 0 to 72 hours, the measurement data is then made into a curve with the x axis as time (t) and y as the OD value (Farisna & Zulaika, 2015).

5. Antimicrobial Activity Assay

The agar diffusion method (Kirby and Bauer disc diffusion) was used to carry out this test. The inhibitory activity was tested against *Klebsiella* sp., *Staphylococcus* sp., which were used as test microbes. To test antimicrobial activity, 6 mm paper discs were used. One *Klebsiella* ose, and *Staphylococcus* sp. that has been inoculated is taken \pm 1 ose and then diluted with sterile 0.9% NaCl solution until it has turbidity in

accordance with Mc standards. Farland 0.5 (10^8 CFU/ml). Then suspend the pathogenic microbes with sterile cotton buds into a petri dish containing MHA media using the streak plate method, then place a paper disk soaked with *Bacillus* bacteria on top, then incubate at 37 °C for 24 hours in this test using antibiotic control (Budianto & Suprastyani, 2017).

Results and Discussion

1. Resistance of *Bacillus* sp. Aceh's Krueng River Against Fe

The resistance test results showed that the isolate *Bacillus* sp. resistance to Fe metal as indicated by the growth of *Bacillus* sp. around the paper disk containing Fe. Colony morphology and resistance test results of *Bacillus* sp. isolates, can be seen in figure 1.



Figure 1 Initial isolate of *Bacillus* sp. (A) and Resistance Test Results *Bacillus* sp. Against Fe with concentrations of 2000 ppm and 2500 ppm (B).

Table 1 Resistance Test of *Bacillus* sp. Against Fe

Replicate	Concentration (mm)									Average (mm)
	U1	U2	U3	U4	U5	U6	U7	U8	U9	
2000 ppm	3,5	2,2	1,46	1,45	2,4	1,2	1,3	1,4	1,5	1,82
2500 ppm	2,7	3,2	1,53	1,56	2,7	4,2	1,7	1,8	1,4	2,31

Inhibition zone measurement criteria are based on Escamilla-Rodríguez *et al.*, (2021) with the inhibition zone produced, bacteria are said to be resistant if the inhibition zone measurement results are <13 mm. Based on the results of the research carried out, testing the resistance of *Bacillus* sp. against Fe metal at a concentration of 2000 ppm showed an average value of 1.82 mm (resistant), and at a concentration of 2500 ppm showed an average value of 2.31 mm (resistant). According to Sulastri *et al.*, (2022) several *Bacillus* species are resistant to Fe metal up to 6000 mg/L on Nutrient Agar and some *Bacillus* species are only able to survive up to 100 mg/L. According to Fahrudin *et al.*, (2020) Bacteria can adapt to extreme environments, bacteria are able

to live in water containing high metal concentrations because bacteria have the ability to detoxify the effects of heavy metals through mechanisms in cells that bind metals.

2. Growth Curve of *Bacillus* sp.

Results of measuring the growth curve of *Bacillus* sp. This was done to see the growth phase of the bacteria. Results of measuring the growth curve of *Bacillus* sp. with a concentration of 3000 ppm. Data from growth curve measurements can be seen in Figure 2 with the highest OD value, namely 3.26 Å.

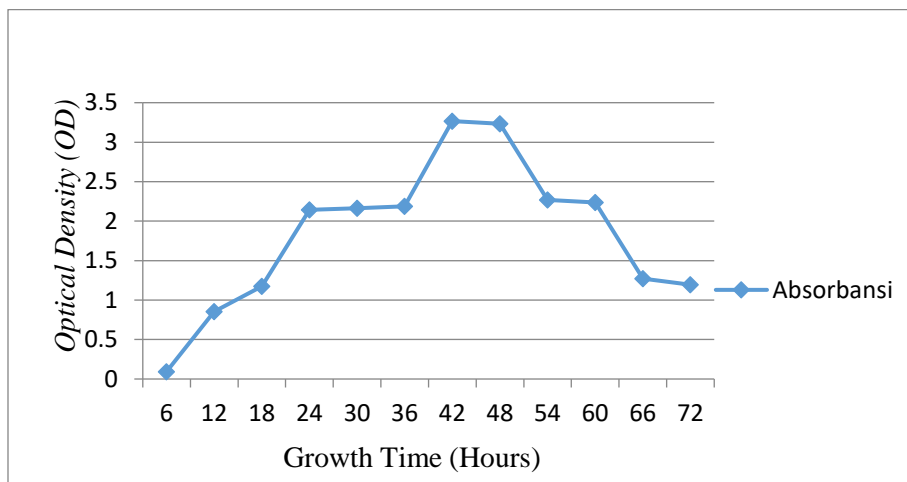


Figure 2 Graph of the Growth Curve of *Bacillus* sp.

Based on the results of the growth of *Bacillus* sp. for 72 hours with measurements every 6 hours. In the first 6 hours the absorbance value was 0.094 and at 72 hours the absorbance value was 1.193. The results of research measuring bacterial growth curves, lag phase (adaptation) of *Bacillus* sp. occurs at hour 0. Then the log (exponential) phase starts from the 12th hour to the 30th hour, with OD values starting from 0.853 Å to 2.162 Å. The log (exponential) phase is the phase needed by microbes to carry out cell division or cell multiplication, which is called generation time, pH, temperature, genetic characteristics and nutrients contained in the media influence this phase. Next, the stationary phase starts from the 30th hour to the 42nd hour with an OD value of 2.162 Å to 3.267 Å and the mortality phase (death) at the 48th hour with an OD value of 3.235 Å. The OD value with the addition of Fe metal is the highest, namely 3.26 Å, while for *Bacillus* without metal, namely 0.5-0.6 Å, this could be because *Bacillus* is resistant to Fe metal and Fe metal in certain concentrations is needed by microbes in cellular respiration (Rahmawati & Zulaika, 2021).

3. Characteristics of Pathogenic Bacteria from the Krueng River, Aceh

The isolation results from Krueng Aceh water samples showed that 8 isolates were classified as gram-negative bacteria and 6 isolates were classified as gram-positive bacteria which were marked in red and purple or blue, which can be seen in Figure 3.

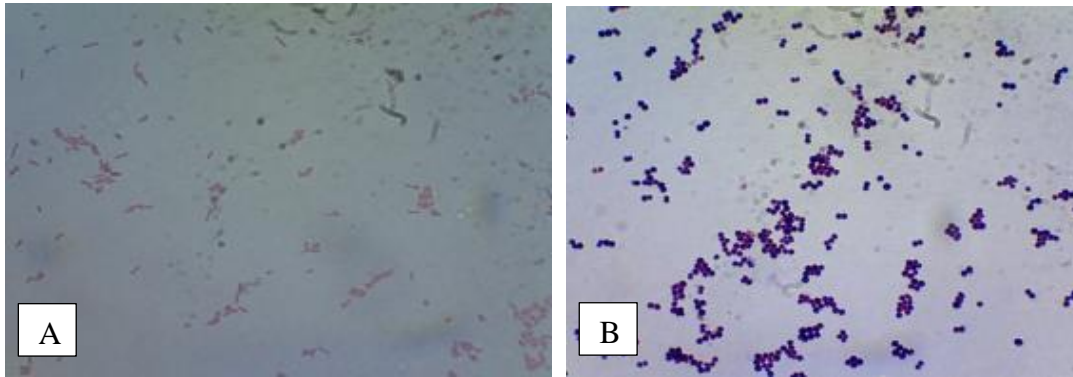


Figure 3 Negative Gram Staining Test (A) and Positive Gram Staining (B)

Table 2 Data on Macroscopic and Microscopic Genus of Bacteria from Various Sources

Macroscopic	Genus of Bacteria		
	<i>Klebsiella</i> sp. (CL1,CL2,CL4,CL7)	<i>Enterobacter</i> sp. (CL3,CL5,CL6,CL8)	<i>Staphylococcus</i> sp. (SA1 , SA2, SA3, SA4, SA5, SA8)
Shape	Circular	Circular	Circular
Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Colour	Pink	Pink	Yellow
Microscopic			
Gram	-	-	+
Cell Shape	Bacil	Bacil	Coccus
Glucose	+	-	+
Lactose	+	-	+
Sucrose	+	-	+
Gas	-	-	-
H₂S	-	-	-
Indole	-	-	-
Motility	-	-	-
Citrate	+	+	-
MR	+	+	+
VP	-	-	-
Catalase	+	+	+

Description: Bacterial Isolates that were successfully isolated, Negative (-) and Positive (+)

The research results showed that there were 3 genera of bacteria that had been isolated in the Krueng River, Aceh, namely *Klebsiella*, *Enterobacter*, and *Staphylococcus*. On research Parhusip *et al.*, (2020) stated that the bacteria that had been isolated from the river were *Enterobacter cloacae*, *Pantoea agglomerans*, *Klebsiella pneumoniae*, *Klebsiella grimontii*, and *Shigella flexneri*. *Klebsiella* sp. On EMBA media, it is characterized by pink colonies with flat edges and a diameter of 2-

5 mm. The catalase test gave positive results and the TSIA test also produced positive results for fermenting sucrose, glucose and lactose. *Enterobacter* sp. has pink colonies on EMBA media with a diameter of 2-3 mm and a convex elevation (Krisnawati et al., 2022).

Staphylococcus sp. on MSA media it has a round, yellow colony shape with flat edges. These bacteria can be found alone or in groups and form clusters like grapes. This bacteria shows positive results on the catalase test, and is facultative anaerobic in nature. The *Staphylococcus* genus is found living on the skin and glands, as well as on the mucous membranes of humans, birds and mammals as opportunistic bacteria. In nature, these bacteria are found in water, soil, plant surfaces and food products (such as uncooked egg yolks) and can cause disease in humans (Nainggolan *et al.*, 2018).

4. Antimicrobial Activity of *Bacillus* sp. Against Pathogenic Microorganisms

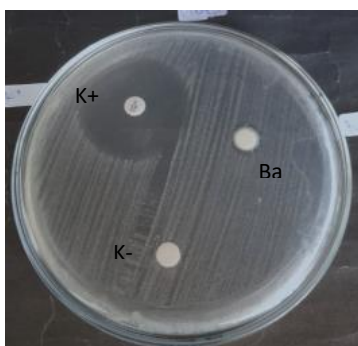


Figure 4 Antimicrobial Activity Test Results for *Bacillus* sp. Against Pathogenic Microorganisms

Table 3 Antimicrobial Activity Test Results for *Bacillus* sp. Against Microorganisms Pathogen

Phatogen Isolate	Antimicrobial	Average (mm)	Inhibition Zone Criteria
<i>Klebsiella</i> sp.	Control (-)	0	Weak
	Control (+)	18,38	Strong
	<i>Bacillus</i> sp.	2,71	Weak
<i>Staphylococcus</i> sp.	Contol (-)	0	Weak
	Control (+)	24,69	Very Strong
	<i>Bacillus</i> sp.	0,51	Weak

Description: weak (<5 mm), moderate (5-10), strong (>10-20 mm) and very strong (>20-30 mm).

Inhibition zone measurement criteria are based on Datta *et al.*, (2019) with a diameter of the inhibition zone it is said to be weak if it shows results <5 mm and it is said to be very strong if it shows results >20-30 mm. In testing the antimicrobial activity of *Bacillus* sp. against *Klebsiella* sp. produces an inhibition zone in the weak category, namely 2.71 mm. The small diameter of the inhibition zone can be caused by the influence of the bacterial cell membrane which functions as a barrier due to the presence of lipids and high permeability. The phosphatidylethanolamine content in Gram-negative bacteria is thought to be the cause of reducing the sensitivity of bacteria to antibacterial compounds to the point that this concentration is not categorized as

strong in inhibitory activity. (Ambarwati & Ibrahim, 2021). In testing the antimicrobial activity of *Bacillus* sp. against *Staphylococcus* sp. produces an inhibition zone in the weak category, namely 0.51 mm. The weak diameter of the inhibition zone can be caused by Gram-positive bacteria having thicker cell walls so antimicrobials will find it difficult to penetrate the cell walls (Datta *et al*, 2019).

Conclusion

Bacillus sp. resistant to Fe metal at concentrations of 2000 ppm and 2500 ppm, with the lowest value at 2500 ppm, namely an average of 2.31 mm. *Bacillus* sp. those exposed to the heavy metal Fe at a concentration of 3000 ppm entered the lag phase (adaptation), namely at hour 0. Then the log (exponential) phase starts from the 12th hour to the 30th hour, then the stationary phase starts from the 30th hour to the 42nd hour. Pathogenic bacteria from the Krueng River, Aceh, found 14 bacterial isolates, namely 4 genus *Klebsiella*, 4 genus *Enterobacter* and 6 genus *Staphylococcus*. Antimicrobial Activity of *Bacillus* sp. which was tested on pathogenic microbes, namely *Klebsiella* sp., and *Staphylococcus* sp. has an inhibitory power in the weak category, namely 2.71 and 0.51.

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