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Optimization Of Antibiotics Production In Bacillus Subtilis Soil Strain G2b Against Staphylococcus Aureus And Escherichia Coli

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Abstract – Bacillus subtilis strain G2B is a potential bacterium derived from anchovy (Stolephorus sp.) processing wastewater. This study aims to analyze the antimicrobial activity of Bacillus subtilis strain G2B in the production of antibiotics against Staphylococcus aureus and Escherichia coli test bacteria. The method used in this research is a survey method, the data is analyzed descriptively quantitatively. The results showed that Bacillus subtilis strain G2B had the ability to produce antibiotics as measured by its antibiosis index, which was 12 mm against Staphylococcus aureus bacteria and 13 mm against Escherichia coli test bacteria. The inhibition zone of Bacillus subtilis strain G2B against the test bacteria belongs to the strong category.

Keywords – Production, Antibiotics, Bacillus subtilis strain G2B, Waste

I. INTRODUCTION

Anchovy (*Stolephorus* sp.) is a commodity that is very popular with the people of Padang City. The content of amino acids and very high water content, which is about 80% of their body weight, makes fish quickly degrade. This is caused by decomposing microorganisms such as bacteria, mold, and yeast. Therefore it is necessary to process fish so that the fish quality is maintained and can be stored for a long time (Yusra and Efendi, 2010).

One of the fish processing centers in Padang City is the Pasie Nan Tigo Fisheries Processing Center (SP3N) Padang City. SP3N is located in the coastal area of Pasie Nan Tigo Village, Koto Tangah District, Padang City. Fish that are usually processed are anchovies (Stolephorus sp.) into dried anchovies (fresh and salted) (Yusra et al., 2019). The processing of dried anchovies at SP3N is carried out systematically. However, they do not yet have an environmental impact analysis (AMDAL) so that the waste is not managed properly. Fish processing liquid waste consists of fish washing and boiling waste. Where the waste still contains pieces of fish bodies and fish blood which are supporting factors for microbial life.

One of the many microbes found in waste is bacteria from the genus Bacillus. Bacteria from the Bacillus genus are gram-positive bacteria that have the ability to produce antimicrobial compounds in the form of antibiotics, proteinases and bacteriocins (Setiaji, 2019). Antibiotics are chemical substances produced by microorganisms that inhibit the growth (bacteriostatic) or kill (bactericidal, virucidal, fungicidal) of other microorganisms.

Research in the search for antibiotic-producing bacteria has been carried out before, such as: identification of antibiotic-producing bacteria from mangrove ecosystems in Dumai (Nursyirwani et al., 2019), isolation of antibiotic-producing microbes

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from the sand of Lemo-Lemo Beach, Bulukumba Regency (Handayani, 2020) and the potential and antibiosis bacteria character of beef slaughterhouse waste (Defnur et al., 2019). However, optimization of antibiotic production from fish processing wastewater has never been carried out. Based on this description, a research was conducted on Optimizing Antibiotic Production *in Bacillus subtilis soil strain G2B* against *Staphylococcus aureus* and *Escherichia coli*. *Bacillus subtilis soil strain G2B* isolate is a potential bacterial isolate obtained from fish washing wastewater when processing anchovies (*Stolephorus* sp.) into dried anchovies. This study aims to analyze the antibiotic activity of *Bacillus subtilis soil strain G2B*, so that it is expected to be used as an antibiotic candidate against *S. aureus* and *E. coli*.

II. Research Methodology

a. Bacterial isolate Bacillus subtilis soil strain G2B,

This study used a potential bacterial isolate of *B. subtilis soil strain G2B*, which was isolated from anchovy (*Stolephorus* sp.) processing wastewater. The bacterial isolates were cultured in sodium agar (NA).

b. Antibiosis Test of Bacillus subtilis soil strain G2B Against Test Bacteria

Bacterial isolate *B. subtilis soil strain G2B* was cultured and incubated at 37°C for 24 hours. Then centrifuge the cultured *B. subtilis soil strain G2B* bacteria. Next, apply the suspension of *Staphylococcus aureus* and *Eschericia coli* test bacteria to the surface of the NA medium until smooth, then leave for 15 minutes at room temperature. Paper discs that have been smeared with a suspension of antibiosis bacteria are placed aseptically on the surface of the medium. Next, incubate at 37°C for 24 hours. Antibacterial activity is indicated by the area of the clear zone formed around the colony.

c. Optimization of Bacillus subtilis soil strain G2B Bacterial Growth

Take 1 ose of bacterial culture grown in 10 ml of GP Broth, then incubate at different times (0 hour, 4 hours, 8 hours, 12 hours, 16 hours....60 hours and observe the turbidity using a spectrophotometer with a wavelength of 600 nm (Axelsson, 2004).

d. Bacterial characterization of *Bacillus subtilis soil strain G2B*

Tests for optimizing the formation of secondary metabolites were carried out at several different variations of pH, temperature and salt content. Take 1 ose of potential bacterial isolates and grow them in 10 ml of GP Broth which has been adjusted for pH, temperature and salt content by incubating for 24 hours at the specified temperature. Then check the growth medium. The presence of microbial activity was indicated by the turbidity of the GP Broth media (Nofisulastri *et al.,* 2006).

III. III. RESULTS AND DISCUSSION

a. Bacterial Antibiosis Test *Bacillus subtilis soil strain G2B* Against Test Bacteria

The antibiosis test was carried out to observe and measure the inhibition zone of bacterial isolates from fish processing wastewater against the test bacteria. According to Surdjowardjojo et al., (2015) there are three categories of inhibition zones, namely inhibition zones less than 5 mm (≤5mm) including the weak category, inhibition zones in the range of 6-10 mm including the medium category and those in the range of 11-20 mm including into the strong category. The antibiosis activity of the bacterial isolate Bacillus subtilis soil strain G2B against the test bacteria can be seen in Figure 1.

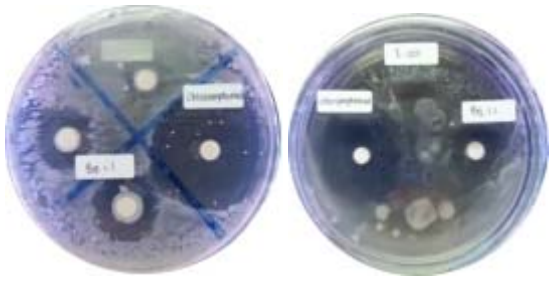

Figure 1. Antibiosis Test of Bacterial Isolates with Test Bacteria

The antibosis test was indicated by the presence of a halo zone around the *Bacillus subtilis soil strain G2B* bacteria colony. The halo zone formed around the bacterial colony of *Bacillus subtilis soil strain G2B* is 12mm against *Staphylococcus aures* and

13mm against *Eschericia coli* test bacteria. This stated that *Bacillus subtilis soil strain G2B* bacteria belonged to the strong category of bacteria. The formation of a halo zone indicates that the antagonist bacteria produces antibiotics. Antibiotics are classified as secondary metabolites produced by endophytic bacteria and antagonists in metabolic pathways and by enzymes that are not needed for the growth and maintenance of plant cells (Rifai, 2020).

This study is in accordance with Defnur *et al.*, 2019 which stated that bacteria isolated from beef slaughterhouse sewage had a free zone diameter (halozone) for Staphylococcus aureus bacteria in the range of 5-20 mm. Zafrana *et al.*, (2021) also found four bacterial isolates isolated from beautiful hybrid grouper larvae that produce antibiotics that have clear zones ranging from 19.54 mm – 22.31 mm. then 14 bacterial isolates isolated from Thai traditional fermented fish (Plasom) found that isolate LPS17 produced the widest inhibition zone against *S. aureus,* which was 38.3 mm, and isolate LPS04 showed the widest inhibition zone against *E. coli*, which was 62 mm.

b. Optimization of *Bacillus subtilis soil strain G2B* Bacterial Growth

Bacteria need generation time in the growth process. Generation time is needed to be able to grow and multiply until the number of each generation is twice the previous population (Fardiaz, 1989). The growth rate of *Bacillus subtilis soil strain G2B* was measured from 0 to 64 hours as shown in Figure 2.

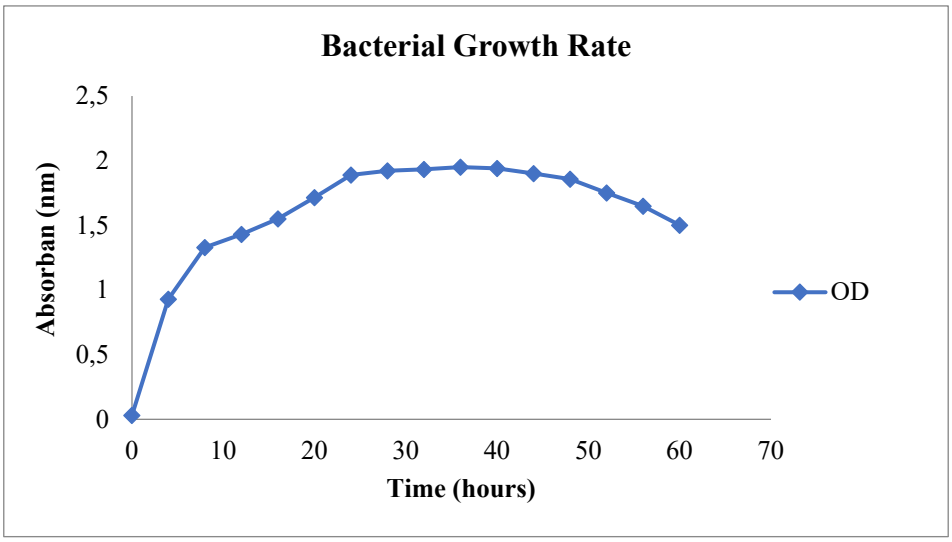

Figure 2. Growth curve of *Bacillus subtilis soil strain G2B* Bacterial Isolate

During 60 Hours Incubation Measured by Optical Density (OD)

The growth of microorganisms has four curve phases, including the lag phase, log phase, stationary phase and death phase. In each phase the growth of bacteria is influenced by several factors such as growth medium and environment, nutrients, temperature, and others (Cappuccino and Sherman, 2014). The maximum growth rate of *Bacillus subtilis soil strain G2B* bacteria was obtained at 36 hours. Bacterial activity increases periodically starting at the 4th hour until the 36th hour, then the curve will decrease. Secondary metabolites are produced maximally during the exponential phase until the beginning of the stationary phase. Secondary metabolites occur during bacterial growth which are antibacterial substances and are synthesized directly on ribosomes. Diaz (1993) said that the best production of secondary metabolites occurs at the beginning of the stationary phase or at the end of the exponential phase. Production of secondary metabolites produced will follow the pattern of primary metabolites.

The growth method of *Bacillus subtilis soil strain G2B* bacteria is almost the same as the growth of bacteria isolated from fermented mackerel (*Scomberomorus guttatus*) where the production of secondary metabolites is formed from the 6th to 22nd hour (Yusra, 2014). Research by Torkar and Matijasic (2003) also stated that the bacterium *Bacillus cereus* derived from milk and its processed products found that bacteriocin production entered a stationary phase after 10-16 hours of incubation.

c. Bacterial characterization of *Bacillus subtilis soil strain G2B*

Characterization of *Bacillus subtilis soil strain G2B* bacteria was carried out to determine the stability and activity of the bacteria in various conditions of pH, temperature and salt content so that they could be used as antibiotic candidates. pH can affect the speed of enzyme activity in catalyzing a reaction in bacteria (Waluyo, 2007). In this study, the bacteria *Bacillus subtilis soil strain GB2* could grow optimally in the range of pH 6 to pH 8. This stated that the bacteria *Bacillus subtilis soil strain G2B* belonged to mesophilic bacteria because it can grow at pH 5.5 – 8. This is also in line with the research of Periadnadi *et al.,* (2021) which stated that the bacterial isolate LRPS 8 isolated from beef slaughterhouse waste was a mesophilic class of microbes.

In addition to pH, the speed of bacterial enzymatic reactions is also affected by temperature. The effect of temperature on microbial growth is due to the fact that temperature affects the activity of enzymes that catalyze biochemical cells in microbial cells (Yusra, 2014). The temperature range for bacterial growth is divided into minimum temperature, optimum temperature, and maximum temperature (Lay, 1994). Based on the results of research on Bacillus subtilis soil strain G2B bacteria, it is classified as the optimum mesophilic bacteria because it grows optimally in the temperature range of 30oC - 40oC. According to Hatmanti (2000) bacteria from the Bacillus group can grow at temperatures of 10-50°C. Hutabarat and Evans (2012) also stated that the optimum temperature range for the life of microorganisms is 25-32°C.

One important factor that can affect the growth of microorganisms is the salt content. Bacteria that are able to live at high salt levels are called halophilic bacteria. Halophilic bacteria can grow optimally at certain minimum NaCl concentrations (Fardiaz, 1989). Based on research results, *Bacillus subtilis soil strain G2B* can grow well at a salt content of 25 – 75 ppt. This research is in line with the discovery of bacteria that are able to grow at a salt concentration of 25% from fermented anchovies (anchovy-jeot) products originating from Korea. Bacteria *Bacillus* sp. KYJ 968 is able to grow up to 15% NaCl concentration (Ha *et al.,* 2002). Nur (2019) obtained four isolates of protease-producing halophilic bacteria isolated from Bledug Kuwu, Central Java. And also found 8 isolates of halophilic bacteria from sediments and marine waters of Bledug Kuwu, Grobogan Regency (Sabdaningsih and Arina, 2020).

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IV. CONCLUSION

Bacillus subtilis soil strain G2B bacteria has the ability to become a candidate for producing antibiotics through an antibiosis test of 12 mm against Staphylococcus aureus bacteria and 13 mm against Escherichia coli test bacteria. The exponential phase begins at the 4th to 36th hour. *Bacillus subtilis soil strain G2B* bacteria belong to mesophilic bacteria at pH 5.5 – 8, optimum mesophilic at 30ºC - 40ºC and halophilic bacteria at salt content of 25 – 75 ppt.

REFERENCES

[1] Axelsson, L., 2004. Lactic Acid Bacteria: Classification and Physiology. In: Salminen, S. von Wright, A. and Ouwehand A. (Eds.), lactic acid bacteria: microbiological and functional aspects. 3rd rev. and exp. ed. Marcel Dekker, Inc., New York, pp. 1-66.

[2] Cappuccino, J. G and N. Sherman 2014. Microbiology a Laboratory Manual 10th Ed. San Francisco : Pearson Education, Inc. Publishing as Benjamin Cummings.

[3] Defnur. A. 2019. Isolation, Potential and Characterization of Antibiosis Bacteria From Cattle Slaughterhouse Wastewater Against *Staphylococcus aureus* Rosenbach Bacteria. (Thesis) Faculty of Mathematics and Natural Sciences, Andalas University.

[4] Diaz, J. R., 1993. Plantaricin S And T, Two New Bacteriocin Produced By Lactobacillus Plantarum LPC010 Isolated From A Green Olive Fermentation. *Journal of Applied Environmental of Microbiology*, 59:1416-1429.

[5] Fardiaz, S. 1989. Food Microbiology. Bogor: Inter-University Center.

[6] Ha, Y. M., Y. H. Park and Y. J. Kim., 2002. A taxonomic study of Bacillus sp. isolated from Korean salt fermented Anchovies. *Molecular Biology Today*, 3(1): 25-29.

[7] Handayani. 2020. Isolation of Antibiotic-Producing Microbes from Lemo-Lemo Beach Sand, Bulukumba Regency in Inhibiting Several Pathogenic Bacteria. *Journal of Health*, 13(1):21-27.

[8] Hatmanti, A. 2000. Introduction of *Bacillus* spp. Jakarta: Research and Development for Marine Environment LIPI.

[9] Hutabarat, S. and Evans. 2012. Introduction to Oceanography. Jakarta : UI-Press.

[10] Lay, B. W. 1994. Microbial Analysis in the Laboratory. Jakarta: Raja Grafindo Persada.

[11] Nofisulastri, Z., Bachruddin and E. Harmayanti, 2006. Production and extraction of antibacterial bacteriocin from *Pediococcus* sp. NWD 015. Indonesian *Journal of Biotechnology*, 11(2): 921-927.

[12] Nur, F. 2019. Isolation and Characterization of Halophilic Microbes Producing Extracellular Proteases from Bledug Kuwu Mud, Central Java. (Thesis). Department of Chemistry FMIPA, State University of Malang

[13] Nursyirwani, Effendi. I and Desi, M. S. 2019. Molecular Identification of Antibiotic-Producing Bacteria from Extreme Microhabitats in Mangrove Ecosystems and Their Activities Against Pathogenic Bacteria (*Vibrio alginolyticus*). *Journal of Fisheries and Maritime Affairs*, 9(2): 137-150.

[14] Periadnadi, Nurmiati and H. A. Defnur. 2021. Optimization of Antibiotic Production On Various Protein Substrates Against Staphylococcus Aureus And Escherichia coli. *International Journal of Progressive Science and Technologies (IJPSAT)*. 28(2): 169-173.

[15] Rifai. M, H. Widowati and A. Sutanto. 2020. Synergism and Antagonism of Several Types of Consumed Bacterial Isolates. *Journal of Biolova* 1(1). 21-26.

[16] Sabdaningsih. A., and Arina T. L. 2020. Isolation and Morphological Characterization of Halophilic Bacteria from Bledug Kuwu, Grobogan Regency. *Journal of Biomes*, 22(1): 46-52.

[17] Setiaji, J. 2019. DNA Analysis of Marine Heterotrophic Bacteria That Are Antagonists Against Pathogenic Bacteria in Fish. *Proceedings of the Seminacle*, 1(1): 17-27.

[18] Surdjowardjojo. P., Susilorini T. E., Sirait G. R. B. 2015. Inhibition of Decoction of Manalagi Apple Skin (Mulus cylvertrs Mill) on the Growth of *Staphylococcus aureus* and *Pseudomonas* sp. *Journal of Tropical Livestock*. 16(2): 40-48.

[19] Torkar. K.G and B.B. Matijasic. 2003. Partial Characterization Of Bacteriocins Produced By Bacillus Cereus Isolates From Milk And Milk Products. *Journal of Food Technology Biotechnology*, 41(2): 121–129.

[20] Yusra and Y. Efendi . 2010. Fundamentals of Fishery Product Technology. Padang: Bung Hatta University Press.

[21] Yusra, Y. Efendi and A. Mardiah. 2019. Training on Fish Handling and Processing in Pasia Nan Tigo Village, Koto Tangah District, Padang City. *Vocational Journal*, 3(2):105-111.

[22] Yusra., F. Azima,. Novelina and Periadnadi. 2014. Antimicrobial Activity Of Lactic Acid Bacteria Isolated From Budu Of West Sumatra To Food Biopreservatives. *Pakistan Journal of Nutrition,* 12(7): 628- 635.

[23] Zafrana, S. Ismia , I. Mastutia and K. Mahardika. 2021. Isolation and Characterization of Bacteria Isolated from Larvae of Beautiful Hybrid Grouper Fish Affected by Stunted Tail Disease. *Journal of Fisheries and Marine Research*, 4(2):194-205.