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Optimization Of Antibiotic Production On Various Protein Substrates Against Staphylococcus Aureus And Escherichia Coli

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***Abstract*— Optimization has been carried out on the protein substrate, isolates of Slaughterhouse Waste. This study aims to determine the best protein substrate for potential antimicrobial isolates (*Bacillus,* Isolate Slaughterhouse Waste) in the production of antibiotics against the test bacteria *S. aureus* and *E. coli*. This study used an experimental method to optimize each of the different protein substrates in the production of antibiotics from potential isolates from the Slaughterhouse Waste tract against *S. aureus* and *E. coli*. From the results of the study, skim milk was obtained as the best substrate because it had the highest *Halozone* diameter of 15 mm against the test bacteria *S. aureus* and 9 mm against *E. coli*. Inhibition against *S. aureus* was strong, while for *E. coli* it was moderate.**

***Keywords*— Antibiotics, Genus Bacillus, Skimmed Milk, Substrate Optimization.**

I. INTRODUCTION

Infectious diseases are caused by infection, infectious diseases develop due to many treatments of resistant infectious bacteria. Microorganisms that have the potential to produce antibiotics are obtained from soil microbes and manure. One of the dirty places where antibiotic candidate bacteria are likely to be found is in the Slaughterhouse Waste channel. In this waste channel, there is waste from the slaughter of cows in the form of blood splashes, the rest of the contents of the stomach of the cow and the crust of the cow slaughter that sticks around the canal. This waste channel has the potential to support microbial life, because it is the habitat of a microbial consortium.

Various studies on the search for antibiotics have been carried out but the optimization of antibiotic production from dirty places has never been reported. Several studies looking for antibiotics, among others, research Defnur (2019) found seven bacterial isolates that had antibacterial potential against *S. aureus* bacteria isolated from the Slaughterhouse Waste tract, the average isolates found were from the genus *Bacillus*.

Norris et al. (1981); Claus and Barkeley (1986) in Hatmanti (2000) stated that bacteria of the genus *Bacillus* are gram-positive bacteria and have physiological properties that have different abilities, including being able to degrade organic compounds such as protein, starch, cellulose, hydrocarbons and agar, being able to produce antibiotics, play a role in nitrification and denitrification, Nitrogen (N) fixing, Selenium oxidizing, Manganese (Mn) oxidizing and reducing agents and are chemolithotrophs, aerobic or facutative anaerobes, acidophilic or alkaliphilic, psychopriphilic, or thermophilic.

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Aminy's research (2019) found ten isolates of antibiosis bacteria from Chicken Slaughterhouse Waste. Further research by Adriani and Tulak (2013) found two bacterial isolates capable of fighting *E. coli* and *S. aureus* bacteria, from cattle farm soil.

Based on this description, a research was conducted on the Optimization of the Use of Various Protein Substrates in the Production of Antibiotic Filtrate Against Test Bacteria. The bacterial isolates used in this study were potential isolates from the Slaughterhouse Waste channel, with values classified as gram-positive bacteria with bacilli, motile, sub-terminal endospores and catalase negative cell shapes. Optimization in the form of protein substrates because the isolates came from the *Bacillus* genus which produces antibiotics from polypeptide amino acids (such as Skim Milk, Flour Soy Milk, Egg Yolk and Peptone). This study aims to obtain the best protein substrate for potential isolates in the production of antibiotics against *S. aureus* and *E. coli*

II. Research Methodology

This study used experimental methods in optimizing each different protein substrate in the production of antibiotics against *S. aureus* and *E. coli*.

1. Preparation Stage

a. Equipment Sterilization

All tools and materials to be used are sterilized using an autoclave at 121 ^{O}C, 15 lbs pressure for 15-20 minutes.

b. Antibiotic Medium

Antibiotic medium is a medium that can produce antibiotics. Several bacteria belonging to the genus Bacillus can produce antibiotics of the Gramicidine, Tyrocidine, Polymyxine and Bacitracine types. Antibiotics from the genus *Bacillus* can be produced with a medium containing amino acids, this is because the antibiotics Gramicidine, Tyrocidine, Polymyxine and Bacitracine are derived from amino acids of the polypeptide group (Hansel, 1980).

The antibiotic medium used contained glucose plus peptone, as well as several different substrates used for the production of antibiotics, namely Skim Milk, Flour Soy Milk, Egg Yolk, Peptone. Various different substrates are made with the following composition:

a. Skim Milk

This substrate is made with a composition of 5 g of skim milk powder in 50 ml of aquadest, heated while stirring until homogeneous and sterilized.

b. Flour Soy Milk

This substrate is made with a composition of 10% by volume (5 g of soy milk flour in 50 ml of aquadest), heated while stirring until homogeneous and sterilized.

c. Egg Yolk

This substrate is made with a ratio of the composition of egg yolk and aquadest 1: 3, then heated until homogeneous and sterilized.

d. Peptone

This substrate was made with a composition of 10% by volume (5 g of peptone in 50 ml of aquadest), then heated with stirring until homogeneous and sterilized.

c. Preparation of Test Bacterial Suspension

Test bacteria that have been rejuvenated are taken one needle loop and inoculated in sterile distilled water until a turbidity equivalent to *Mc. Farland's 0.5* is obtained.

2. Implementation Stage

a. Main Starter Preparation

A potential isolate starter was made by taking 1 ose of *Bacilus* Isolate Slaughterhouse Waste scouring, put into a liquid culture medium containing of 1 g glucose plus 1 g peptone in 100 ml of solution (modified Meevootisom, *et. al*, 1983), vortexed and incubated at room temperature 37 ^{O}C for 24 hours. The result of the starter that has been incubated is called starter I. Then starter II is made by taking starter I as much as 10%, put into an erlenmeyer containing a liquid culture medium containing of 1 g glucose plus 1 g peptone and the volume is made up to 100 ml (modification of Meevootisom, *et. al,* 1983) were incubated in a shaker at room temperature (28 ^{O}C) at 120 rpm for 24 hours.

c. Antibiotic Filtrate Production

This was done by taking 10 ml of starter II and putting it into various substrates consisting of Skim Milk, Flour Soy Milk, Egg Yolk, Peptone incubated in a shaker at room temperature (28 ^{O}C) at a speed of 120 rpm. The shaker results were centrifuged at 10.000 rpm for 10 minutes to form a supernatant (antibiotic filtrate).

d. Optimization of Various Protein Substrates

This treatment was carried out by dipping the disc paper into the antibiotic filtrate, planted in a petri dish containing *Nutrient Agar (NA)* medium and scratched with *S. aureus* and *E. coli* test bacteria and then incubated. This treatment was repeated 3 times. The diameter of *Halozone* of the formed antibiotic filtrate was measured, presented in the diagram.

III. Result And Discussion

The test results for measuring the diameter of bacterial *Halozone* on each protein substrate from the test are presented in the diagram.

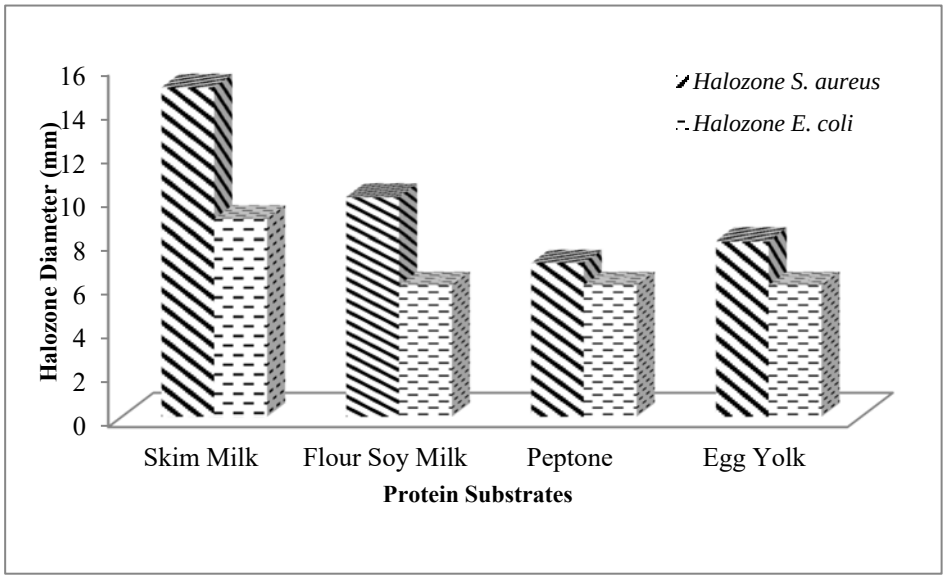

Figure. Diameter Diagram of *Halozone* From Several Protein Substrates Against Test Bacteria.

Based on the diagram, it can be seen that the antibiotic filtrate *Halozone* was formed against the test bacteria. This indicates that some protein substrates can form antibiotics in bacteria of the genus *Bacillus*. *Halozone* formed from the absorption area of the antibiotic filtrate on the paper disc can fight 2 test bacteria.

The test bacteria *S. aureus* showed that the Skim Milk had the highest antibiotic filtrate *Halozone* diameter value of 15 mm. Next, successively followed by Flour Soy Milk; Egg Yolk and Peptone by 10 mm; 8 mm and 7 mm. The control used the antibiotic Cloramphenicol with a *Halozone* diameter value of the antibiotic filtrate of 32 mm against the test bacteria *S. aureus*.

Meanwhile, the test bacteria for *E. coli* showed that the Skim Milk substrate had the highest antibiotic filtrate *Halozone* diameter value of 9 mm. In Peptone, Flour Soy Milk and Egg Yolk could not form *Halozone* antibiotic filtrate, but only the diameter of the paper disc formed was 6 mm where this diameter was obtained by adding 1 mm (because it gave the diameter

and did not grow the test bacteria) with 5 mm diameter of the disc paper and the surface disc paper that is clean from the test bacteria.

This indicates that there is no ability of Peptone, Flour Soy Milk and Egg Yolk substrates in inhibiting the growth of *E. coli* test bacteria. The control used the antibiotic Chloramphenicol with a *Halozone* diameter of the antibiotic filtrate of 38 mm against the test bacteria *E. coli*.

From the analysis of the data obtained, when compared to the ability of the bacteria to inhibit the growth of the test bacteria, the antibiotic properties of this potential isolate were more able to fight the *S. aureus* test bacteria than the *E. coli* test bacteria. The high and low diameter of the *Halozone* formed was caused by the influence of the different protein substrates for the growth of the antibiotic filtrate inhibiting the growth of the test bacteria and also due to the difference in the protein content of each substrate so that the amino acids contained in the protein substrate were also different.

The protein content of each protein substrate can be affected by the volume of different substrates. The 1000 g packaged Skim Milk has a protein content of 0.8 %, while the Skim Milk volume used for this test of 5 g has a protein content of 0.004%. Furthermore, the 200 g packaged Flour Skim Milk has a protein content of 7.5%, while the Flour Skim Milk volume used for this test of 5 g has a protein content of 0.18%. Furthermore, the Egg Yolk used has a volume of 12.5 g which has a protein content of 2.16% and Peptone used has a volume of 5 g. However, the high protein content cannot guarantee the formation of a large *Halozone* diameter, because the types of amino acids in each protein substrate are different.

Dominant amino acids in Skim Milk can support bacterial growth compared to amino acids on substrates Flour Soy Milk, Egg Yolk and Peptone. Modified from research by Magan, *et al.* (2019) reported serine as the dominant amino acid found in Skim Milk, where the Skim Milk used in this study came from cows that were fed pure dry grass.

The amino acid Serine affects the growth of bacteria. This is supported by the statement of Subramanian, *et al.* (2013) in Klewing, *et al.* (2020) where Serine is an important amino acid because this molecule is not only a building block for protein synthesis but also a nucleotide precursor, phospholipid.

On the other hand, Skim Milk contains protein in the form of casein which is easily digested so that it accelerates bacterial growth so that it can fight *S. aureus* test bacteria compared to *E. coli* test bacteria. This is supported by the statement of Rohan, et al. (2016) stated that based on chemical composition, proteins are classified as simple proteins which when hydrolyzed only produce amino acids and complex proteins which when hydrolyzed will produce amino acids and other compounds.

Casein in milk has four types of polypeptides (αs1-, αs2-, β-, κ-Casein) with several genetic variations. κ-Casein is one of the Casein fractions that affects the shape and stability of milk, so that it functions as a determinant of the size and function of milk granules. κ-Casein protein in Cow's milk is composed of 169 amino acids (Sumantri, *et al.,* 2008).

In Skim Milk, it is able to form *Halozone* diameter so that it has the potential for antibiotics against the test bacteria on the same medium (*Nutrient Agar*). This is in accordance with the statement of Park and Nam (2015) which stated that Cow's milk and colostrum and other milk species are considered the most important sources of natural bioactive components. Once Bioactive Peptides (BPs) are released, they exhibit wide physiological variations in the human body such as digestive, cardiovascular, immune, endocrine, and nervous systems. These functions of peptides in human health and physiology include antihypertensives, antibiotics and antioxidants.

In the test bacteria *S. aureus*, the *Halozone* diameter value of the antibiotic filtrate was higher than the bacteria tested for *E. coli*. The antibiotic properties of this potential isolate were more Bacteriostatic (Inhibiting Growth) in the test bacteria *S. aureus* (gram-positive bacteria) than in the test bacteria *E. coli* (gram-negative bacteria).Chloramphenicol antibiotic control has a broad spectrum, this is because it can kill (Bactericidal) 2 test bacteria.

This is in accordance with the statement of Ganiswarna (1995) that the nature of selective toxicity, antimicrobial inhibits microbes (Bacteriostatic) / Minimum Inhibitory Levels and kills microbes (Bactericidal) / Minimum Killing Levels. Certain antimicrobials bacteriostatic activity may increase to be bactericidal. Antimicrobial properties are different, there are active against gram-positive bacteria and active against gram-negative bacteria.

IV. CONCLUSION

Based on the research that has been done, it can be concluded that the Skim Milk substrate is the best protein substrate for the production of antibiotics from this potential isolate Slaughterhouse Waste against *S. aureus* and *E. coli* bacteria.

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