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THE INFLUENCE OF pH AND TEMPERATURE ON THE ANTIMICROBIAL ACTIVITY OF CELL-FREE SUPERNATANT OF *LACTOBACILLUS PLANTARUM* SN13T ISOLATED FROM STINGLESS BEE HONEY (GALO-GALO)

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ABSTRACT

This study aimed to measure the antimicrobial activity of *Lactobacillus plantarum* SN13T cell-free supernatant against bacterial pathogens at different pH and temperatures. *Lactobacillus plantarum* SN13T was isolated from stingless bee honey (galo-galo). Some pathogenic bacteria were tested, namely *Listeria monocytogenes VTO, Listeria monocytogenes, Listeria innocua, Staphylococcus aureus, Salmonella, Propionibacterium acnes, Pseudomonas, Klebsiella, Acinetobacter baumani,* and *Escherichia coli*. Cell-free supernatant (CFS) *Lactobacillus plantarum* SN13T had antimicrobial activity against pathogenic bacteria. The highest antimicrobial activity of CFS *L. plantarum* SN13T at pH 2 was against Salmonella bacteria (23.9 mm), and the smallest inhibition (10.2 mm) was against *Acinetobacter baumani*. While at pH 4, the highest antimicrobial activity of CFS was found in E.coli with an inhibition zone of 26.9 mm. CFS *Lactobacillus plantarum* SN13T at different temperatures still had antimicrobial activity, even with more than 121°C. CFS Lactobacillus plantarum SN13T produced the highest antimicrobial properties against Pseudomonas, both at 70, 100, and 121°C, while the lowest antimicrobial activity was shown against *Litera monocytogenes* VTO.

Keywords: antimicrobial activity, Cell-free supernatant, *Lactobacillus plantarum* SN13T, pathogenic

INTRODUCTION

Today, consumers start to pay attention to health by consuming food sources that benefit the body. It has also become a priority for the food industry to prevent the infections caused by food sources, especially pathogenic bacteria. Therefore, the demand for functional food is increasing, one of which is food that contains probiotics. To meet the consumer demand, research to explore various natural resources that can potentially have lactic acid bacteria (LAB) as probiotics. Previous studies [1, 2, 3] found LAB naturally found in milk, budu fish, and palm sugar.

Furthermore, LAB that has passed the probiotic selection can be applied to process healthy livestock products. Lactic acid bacterial fermentation is one of food preservation and preservation that has been done for a long time. LAB is essential for manufacturing fermented products such as fermented milk, yogurt, and others [4,5]

In addition to maintaining the digestive system, lactic acid bacteria may lower cholesterol, suppress pathogenic bacteria, enhance immune system function, ease constipation, prevent diabetes, and make lactose more digestible for lactose intolerant individuals.[6]

The ability of these bacteria to play a role in food preservation is attributed to the fact that they may cause pH decrease as a consequence of the production of lactic acid and the production of some antimicrobial agents (such as bacteriocins, organic acids, and hydrogen peroxide. The combination of these factors limits the proliferation of unwanted microorganisms (decay or pathogen-microorganisms). Therefore LAB plays an essential role in food safety [7].

A study was conducted to examine the effect of pH and temperature on the antimicrobial activity of the cell-free supernatant (CFS) Lactobacillus plantarum SN13T. Lactobacillus plantarum SN13T is a lactic acid bacteria isolated from West Sumatra, Indonesia's galo-galo honey (stingless bee). According to [8], Indonesia, as a tropical country, has a high diversity of stingless bees. Most of it produces honey that can be used sustainably for human needs.

MATERIAL AND METHODS

Sampling

The research material is Galo–Galo honey (stingless bees) obtained from the Galo–Galo cultivation farm by Rusdimasnyah, S.TP., MP. The types of pathogenic bacteria are Listeria monocytogenes VTO, Listeria monocytogenes, Listeria innocua, Staphylococcus aureus, Salmonella, Propionibacterium acnes, Pseudomonas, Klebsiella, Acinetobacter baumani, and Eschericia coli. This research was conducted at the Animal Products Technology Laboratory, Faculty of Animal Husbandry, Andalas University, Padang, Indonesia.

Cell-Free Supernatant Preparation.

Ten milliliters of *Lactobacillus plantarum* SN13T culture were incubated for 24 hours at 37°C in 90 ml MRS Broth. Then it was centrifuged at 14,000 rpm for 8 minutes at 4°C. The supernatant was filtered using 0.22 μm of filter membrane [9].

Supernatant Testing of Bacteriocin Antibacterial Compounds

Pathogenic bacteria were cultured at 37°C for 24 hours 0.2% of pathogenic bacteria were put into 20 ml of Nutrient Agar that had been cooled to 50°C and allowed to solidify, then made a well in the hardened media with a diameter of 4 mm using a cock drill. Next, the cell-free

supernatant was put into the hole and incubated for 15 hours. The diameter of the clear zone formed around the well using a caliper.

Characterization of antimicrobial of Cell-Free Supernatant

Effect of pH

4.5 mL of nutrient broth with the pH values of control, 2, and 4 were mixed with 0.5 mL of CFS to test the impact of pH. The mixture was then incubated at 37°C for 30 min. The indicator bacteria were tested using the agar diffusion methods against each of the CFS samples treated at various pH levels [10].

Effect of temperature

The test tube included 4.5 ml of nutrient broth and 0.5 mL of CFS. After that, each test tube was covered in paraffin oil to prevent evaporation before being heated for 10 minutes at various temperatures (control, 70, 100, and 121°C). The preparations, including nutrient broth (4.5 mL) and bacteriocin (0.5 mL), were sealed with non-absorbent cotton, covered with aluminum foil, and heated to 121°C for 10 minutes to test its action at a very high autoclaving temperature. The agar diffusion method was used to determine the antimicrobial activity of the various heat-treated specimens mentioned above [10].

RESULTS AND DISCUSSION

The influence of pH on antimicrobial activity

Lactobacillus plantarum SN13T was obtained from the molecular isolation of gallo-galo honey (*Tetrigona bhinghami*) using 16S rRNA. These bacteria can produce secondary metabolites like bacteriocin, hydrogen peroxide, carbon dioxide, acetaldehyde, and primary antimicrobial metabolites like lactic acid. The cell-free supernatant (CFS) Lactobacillus plantarum SN13T had antimicrobial activity tested against 10 microbial pathogens: *Listeria monocytogenes VTO, Listeria monocytogenes, Listeria innocua, Staphylococcus aureus, Salmonella, Propionibacterium acnes, Pseudomonas, Klebsiella, Acinetobacter baumani*, and *Escherichia coli*. The results of antimicrobial activity testing of Lactobacillus plantarum SN13T with ten types of pathogenic bacteria with three different pH treatments on the supernatant that can be seen in Figure 1 below:

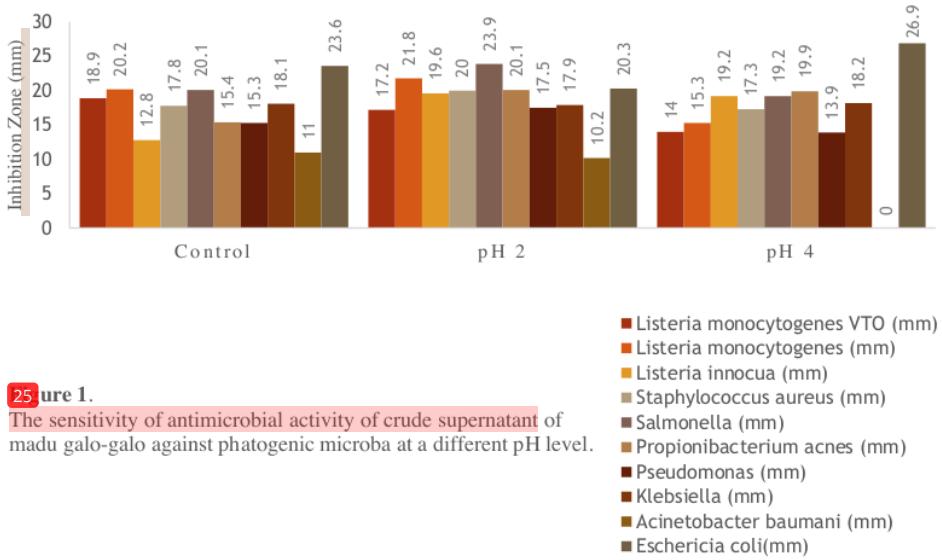

Figure 1.
The sensitivity of antimicrobial activity of crude supernatant of madu galo-galo against phatogenic microba at a different pH level.

Figure 1 shows the antimicrobial activity of CFS at different pH conditions. Antimicrobial activity of CFS L.plantarum SN13T at pH 2 with the highest value was aganst *Salmonella bacteria* (23.9 mm) and the smallest inhibition (10.2 mm) was against *Acinetobacter baumani*. Meanwhile, if the pH was set to higher condition, namely pH 4, the antimicrobial activity of CFS L. plantarum SN13T with the highest inhibition occurred in E.coli with an inhibition zone of 26.9 mm. Still, at this pH, there was no inhibition (0 mm) in *Acinetobacter baumanii*. Antibacterial activity generally increases the acidic pH. It is supported by opinion [11], which states that an acidic pH can increase the interaction of bacteriocins in membrane receptors. More molecules will stick to the cell wall, making the bactericidal molecules stronger.

The difference in antimicrobial activity of CFS L. plantarum SN13T against 10 test bacteria was caused by the ability of the antimicrobial compounds contained in it. It follows opinion [10], which states that various types of antimicrobial produced by lactic acid bacteria are derived from organic acids, diacetyl, hydrogen peroxide, and protein bacteriocins. Furthermore, [12] describes several mechanisms by which bacteriocins kill target bacteria, namely by damaging the cell wall of the target microbe, destabilizing the cytoplasmic membrane, changing the structure of nucleic acids, inhibiting enzyme activity, and inhibiting nucleic acid synthesis.

For CFS L.plantarum SN13T, when compared to the antimicrobial activity in other studies, it showed more significant antimicrobial activity against *Listeria monocytogenes*

bacteria, as seen from the inhibition zone obtained, which was 19.6 mm. Research [13] showed that crude lactic acid bacteria from curd had antimicrobial activity at pH 3 ranging from 1.30 mm-10.03 mm, and at pH 5 ranging from 0.43 mm-13.95 mm.

Both *E. hirae* LD3 and *E. faecium* LR/6 bacteriocins are stable in the pH ranges 2–6 [14] and 2–6 [15], respectively. According to [16], a bacteriocin produced from E. durans 152 maintained all of its anti-listerial action in the pH range of 2–8. Regarding their prospective usage as bio preservatives in food items and fermentation, stable bacteriocins over a broad pH range have a substantial advantage.

The effect of temperature on antimicrobial activity

The results of antimicrobial activity microbial of *Lactobacillus plantarum* SN13T cell-free supernatant against pathogenic bacteria at different temperatures can be seen in Figure 2 below:

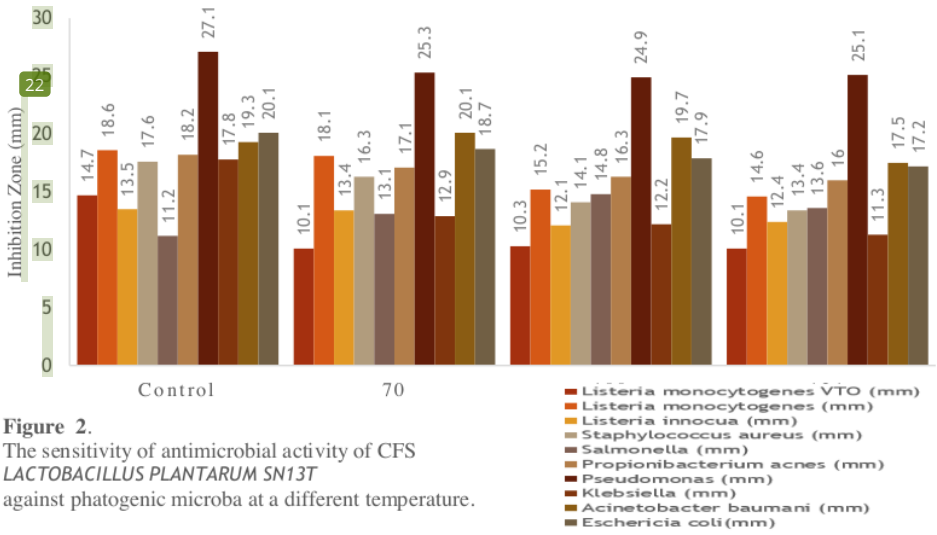

Figure 2.
The sensitivity of antimicrobial activity of CFS *LACTOBACILLUS PLANTARUM SN13T* against phatogenic microba at a different temperature.

Figure 2 showed that CFS *Lactobacillus plantarum* SN13T at different temperatures still had antimicrobial activity, even though the temperature was getting higher (121° C). It is proved by the presence of an inhibition zone formed against all test microbes. CFS *Lactobacillus plantarum* SN13T produced the highest antimicrobial properties against *Pseudomonas*, both at 70, 100, and 121°C compared to control. Meanwhile, the lowest antimicrobial activity was shown against *Litera monocytogenes* VTO compared to the control.

These results align with [13], that isolates isolated from curd had bacteriocin resistance at high temperatures (121° C). Bacteriocins are protein compounds that are biologically active in inhibiting the growth of pathogenic bacteria [17, 18].

They reported by [19] that the antimicrobial activity of *L. plantarum* isolated from cow milk decreased by more than 80% after heating at 121°C. Types of lactic acid bacteria had different bacteriocin stability. It proves that the various strains of *L. plantarum* will affect the characteristics of the plantarcin. Compared with previous studies conducted by [20], the results of this study stated that lactic acid bacteria from buffalo milk had antimicrobial activity against Listeria monocytogenes. Furthermore, [21] stated that the crude bacteriocin Pediococcus acidilactici BK01 from the isolation of tamarind had antimicrobial activity against 2 pathogenic bacteria, namely E. coli O15:H7 and S. aureus ATCC 25923, which were able to survive at a temperature of 121° C.

Figure 2 shows that CFS L.Plantarum SN13T could inhibit the growth of Listeria monocytogenes VTO, Listeria monocytogenes, Listeria innocua, Staphylococcus aureus, Salmonella, Propionibacterium acnes, Pseudomonas, Klebsiella, Acinetobacter baumani, and Eschericia coli. It indicated that the crude bacteriocin inhibitory activity effectively against Gram-positive and Gram-negative bacteria. It is consistent with the view that [22] bacteriocins, an antimicrobial protein substrate that can inhibit sensitive strains, can prevent bacterial infections (gram-positive and gram-negative bacteria).

A bacteriocin that they isolated from E. faecalis EF 478 remained stable following heat treatment at 60°C for one hour. Still, activity dropped at 80°C for one hour and at higher temperatures, that according [23]. According to [24], the inhibitory compound isolated from the E. lactis Q1 strain has a heat tolerance limit of 60°C/30 min or 100°C/15 min. Bacteriocin KT11 could be categorized as a proteinaceous substance due to its thermostability at 121°C for 30 min.

CONCLUSION

Cell-free supernatant (CFS) Lactobacillus plantarum SN13T has antimicrobial activity against pathogenic bacteria. The highest Antimicrobial activity of CFS L.plantarum SN13T at pH 2 was against *Salmonella bacteria* (23.9 mm), and the smallest inhibition (10.2 mm) was against *Acinetobacter baumani*. At pH 4, the highest antimicrobial activity of CFS was found in *E.coli* with an inhibition zone of 26.9 mm. CFS Lactobacillus plantarum SN13T at different temperatures even until 121°Celcius still had antimicrobial activity. CFS Lactobacillus

plantarum SN13T produced the highest antimicrobial properties against *Pseudomonas*, both at 70, 100 and 121ºC, while the lowest antimicrobial activity was shown against *Listeria monocytogenes* VTO.

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