e-ISSN XXXX-XXXX
p-ISSN XXXX-XXXX

UMY UNIVERSITAS MUHAMMADIYAH YOGYAKARTA Unggul & Islami

LEMBAGA RISET DAN INOVASI

UGRACE Universitas Muhammadiyah Yogyakarta Graduate Conference

UNIVERISTAS MUHAMMADIYAH YOGYAKARTA INTERNATIONAL GRADUATE CONFERENCE

Volume 4, Issue 1, August 2024

TRACK CONFERENCE

DiGeS International Graduate Conference on Digital Policy and Governance Sustainability

EBTCoS International Graduate Conference on Engineering, Biotechnology, and Computer Science

SAGE International Graduate Conference on Smart Agriculture and Green Renewable Energy

BioTec International Graduate Conference on Biomedical, Pharmacology, Bioengineering, and Technology

Vol. 4	Iss. 1-2	Yogyakarta 2024	ISSN XXXX-XXXX

 Doi: 10.18196/umygrace.v4i1.664

The Optimization of Temperature to Bacteriocin by *Lactobacillus acidophilus* Against *Methicillin-Resistant Staphylococcus aureus*

Cut Laras Dwi Hendia Putri[1, a)], and Lilis Suryani [2, b)]
[1]Medicine Studies, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Indonesia, 55183
[2]Departement of Microbiology, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183

Email: cut.laras.fkik22@mail.umy.ac.id[a]; lilis.suryani@umy.ac.id[b]

ABSTRACT

Introduction: *Methicilin resistant Staphylococcus aureus* (MRSA) is a pathogenic bacterium that has evolved resistance to antibiotics. MRSA is resistant to the antibiotics methisillin, cephalosporin, and betalaktam. MRSA causes many health problems ranging from lung damage to septicemia. The prevalence of MRSA infections in the world reached 14.69%. *Lactobacillus acidophilus* produces an antimicrobial substance bacteriosine. These bacteriosins have bactericidal and bacteriostatic activity. **Purpose**: The study aims to recognize the influence of temperature on the activity of bacteriosins produced by *L.acidophilus* against MRSA. **Methode**: This research used a quasi-experimental method. *L.acidophilus* reproduced on liquid MRS media for 48 hours. Bacteriosine is obtained by centrifying a MRS broth containing bacteria at a speed of 6000 rpm for 40 minutes. The fluid was tested for its antibacterial effects on MRSA bacteria using Kirby Bauer's method. Bacteriosine is given at different temperatures that is; 40, 60, 80, dan 100 ºC. The diameter of the formed barrier zone is measured in millimeters. Data analysis with ANOVA method. **Finding**: Bacteriosine activity produced by *Lactobacilus acidophilus* against MRSA shows an average inhibition zone diameter of 5.25 mm. The optimum broadest spectrum of antibacterial activity at temperature 60 ºC. **Conclusion**: This research shows that temperature does not affect the antibacterial activity of bacteriosin produced by *L.acidophilus* against MRSA. **Originality/Implication:** The results of this study prove that temperature treatment with various variations does not affect the ability of the bacteriosine produced by *L.acidophilus* to inhibit/kill MRSA.

Keywords: MRSA, Temperature, Bacteriosin, *L.acidophilus*

ABSTRAK

Introduction: *Methicilin resistant Staphylococcus aureus* (MRSA) adalah bakteri patogen yang terbentuk karena resisten terhadap antibiotik. MRSA resisten terhadap antibiotik methisillin, cephalosporin, dan betalaktam. MRSA menyebabkan banyak masalah kesehatan mulai dari kerusakan paru-paru hingga septikemia. Prevalensi infeksi MRSA di dunia mencapai 14.69%. Bakteri *Lactobacillus acidophilus* menghasilkan zat antimikroba bakteriosin. Bakteriosin tersebut memiliki aktivitas bakterisidal dan bakteriostatik. **Purpose**: mengetahui pengaruh suhu terhadap aktivitas bakteriosin yang dihasilkan oleh *Lactobacillus acidophilus* terhadap MRSA. **Methode**: Penelitian ini menggunakan metode quasi-eksperimental laboratorium. *L.acidophilus* ditumbuhkan pada media MRS cair selama 48 jam. Bakteriosin diperoleh dengan cara mensentrifuse MRS cair yang berisi bakteri pada kecepatan 6000 rpm selama 40 menit. Cairan yang terbentuk diuji efek antibakterinya terhadap bakteri MRSA menggunakan metode Kirby Bauer. Bakteriosin diberi pada suhu yang berbeda yaitu; 40 ºC, 60 ºC, 80 ºC, dan 100 ºC. Diameter zona hambat yang terbentuk selama proses pengujian diukur dengan satuan millimeter. Data dianalisis dengan metode ANOVA. **Finding**: Aktivitas bakteriosin yang dihasilkan *Lactobacilus acidophilus* terhadap MRSA menunjukkan rata-rata diameter zona inhibisi sebesar 5.25 mm. Spektrum aktivitas antibakteri terluas pada bakteriosin yang dipanaskan pada suhu 60 ºC. **Conclusion**: Studi ini menunjukkan hasil bahwa suhu tidak mempengaruhi aktivitas antibakteri bakteriosin yang dihasilkan *L.acidophilus* terhadap MRSA. **Originality/Implication:** Hasil penelitian ini membuktikan bahwa perlakuan suhu dengan berbagai variasi tidak mempengaruhi kemampuan bakteriosin yang dihasilkan *L.acidophilus* dalam menghambat/membunuh MRSA.

Keywords: MRSA, Temperature, Bacteriosin, *L.acidophilus*

INTRODUCTION

Methicillin resistant Staphylococcus aureus (MRSA) is a bacterium that is resistant to a variety of antibiotics. The spread of the bacteria can be through direct contact with patients and health workers working at one of the health services (Erlin et al., 2020). MRSA causes inflammation of the skin, soft tissue contamination, bacterial infection, septicemia, toxic shock, and leprosy skin syndrome. The increasing resistance of MRSA to large amounts of antibiotics has caused many losses, including decreased health, economic poverty, and human resource poverty (Abbott et al., 2015). So, an alternative approach is needed to overcome these losses.

The Word of God in the Surah of Yunus 57 "O humanity! Indeed, there has come to you a warning from your Lord, a cure for what is in the hearts, a guide, and a mercy for the believers."

One alternative is to use the bacteriosin compound, which is a protein composition produced by lactic acid bacteria (BAL) (Hernandez et al., 2021). Bacteriosine can be used as a substitute for antibiotics to prevent or treat bacterial infections. Bacteriosine also has several advantages, among others, is non-toxic and easily biodegradable as it is a protein that does not harm the intestinal microflora, is easily digested by enzymes in the digestive tract, and is safe for the environment (Suganya et al., 2013).

Bacteriosin is used as an alternative to antibiotic agents in the prevention or treatment of bacterial infections (Heilbronner et al., 2021). Bacteriosin is effective and selective against food pathogens such as *Staphylococcus aureus*, *Pseudomonas fluorescens*, *P. Aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, and *Listeria monocytogenes*. Bacteriosins produced by *Lactobacillus fermentum* and *Lactocillus acidophilus* have an effect to controlling cephalosporin- resistant *Escherichia coli* infections (Roces et al., 2012).

Bacteriosine acts as a signal peptide to regulate the host's immune response. Does not damage comensal bacteria and is not cytotoxic to eukaryotic cells (Perez et al., 2014). This research was conducted with the aim of identifying the ability of bacteriosins produced by *L. acidophilus* to inhibit the growth of MRSA bacteria at varying warming temperatures. Besides, it is also to obtain the optimal temperature of bacteriosin activity in inhibiting/killing MRSA bacteria.

LITERATURE REVIEW

(1) *Methicillin resistant Staphylococcus aureus* (MRSA)
Bacterial infection of Staphylococcus aureus MRSA, also known as *Methicillin-resistant Staphylococcus Aureus*, is very difficult to treat because the bacteria have immunity to some antibiotic treatments. MRSA bacterial infection has become a global problem that is widespread in several countries. The number of cases itself is still high and continues to rise throughout the world (Almira et al., 2022). Data from the World Health Organization suggests that MRSA is the number two infectious germ that needs to be researched and deepened to make new antibiotics. MRSA infections can cause a variety of diseases, such as skin infections and wounds to lung infections, pneumonia, as well as infections in the bloodstream that can lead to sepsis, even death (Almira et al., 2022). MRSA is considered the leading cause of nosocomial infections worldwide. It was that individuals with MRSA bacterial infections in their bodies were 64% more likely to die than individuals who were infected with bacteria that were not resistant to a variety of drugs. Therefore, the treatment of this MRSA bacterial infection is very important to society (Almira et al.,2022).

(2) *L. acidophilus*
Bacteria of the type *L. acidophilus* were used in this study. Isolate regeneration is done in the MRSA media. Updating and growth in cell proliferation in bacteria is known as regeneration. This is important to do because it will produce new, active bacterial breeds that are ready for an optimal fermentation process (Freire et al., 2015).
The bacteria *Lactobacillus acidophilus* can be used to make probiotics. Because of the bacterial ability to convert sugar into lactic acid, lactic acid bacteria (BAL) are widely usedin the fermentation process to produce lactic Acid (Rahmadi, 2019). With their ability to transform sources of sugar, especially lactose, into lactate acid, *Lactobacillus bacteria* can be distinguished from other acid-producing bacteria (Rahmadi, 2019). Strain *Lactobacillus* has high commercial value due to its high tolerance to acid, yield, and productivity. In addition, the strain of Lactobacillus can be modified to make selective L/D acid lactate (Anggraini, 2019).

(3) Bacteriocins
Bacteriocins are toxic proteins produced by a number of bacteria and certain members of the archaea with the aim of preventing the growth of similar or closely related strains. These proteins are made by ribosomes with antimicrobial capabilities at certain concentrations (Negash & Tsehai, 2020).
Bacteriocins have many benefits that can be exploited. They are active and resistant to extreme heat pressures. Besides, these antimicrobial proteins have no taste, color, or smell. Their protein properties make them easily degraded by proteolytic enzymes, so bacteriosin fragments do not last long in the human body or in nature. This reduces the risk of interaction between target strains and fragmented bacteriosins. Bacteriosine has become a popular ingredient in the food, pharmaceutical, and agricultural industries because of this advantage (Negash & Tsehai, 2020).

METHOD

A. Study design

This research is a laboratory experiment, the research was conducted in May - June 2024 and was located in the Microbiology laboratory of the Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta. This research has obtained permission from the Health Research Ethics Committee of FKIK UMY no. 046/EC-EXEM-KEPK FKIK UMY/V/2024.

B. Samples

The research sample was *Lactobacillus acidophilus* collection from the PAU UGM laboratory on Jl. Teknika Utara, Caturtunggal, Depok, Sleman Regency, Yogyakarta Special Region. The test bacteria used was Methicilin resistant Staphylococcus aureus (MRSA) local isolate from the Microbiology Laboratory of Faculty of Medicine UGM.

C. Tools and Materials

The tools used in this study include: Petri dish (Iwaki pyrex), Ose needle, object glass, test tube, erlemeyer tube (Iwaki pyrex), beaker glass (Iwaki pyrex), autoclave, microscope, incubator, Biological Safety Cabinet, bunsen, refrigerator, centrifugation, water bath and caliper.

The materials used in this study include: Lactobacillus acidophilus isolate, De Man Rogosa and Sharpe (MRS) agar media, De Man Rogosa and Sharpe (MRS) broth media, distilled water, crystal violet, iodine, 95% ethanol, safranin, phosphate spar, NaOH, HCl, Mueller Hinton Agar (MHA), Trypticase Soy Agar (TSA).

D. Research Procedure

1) Rejuvenation of *Lactobacillus acidophilus* bacterial culture
Lactobacillus acidophilus was subcultured on MRS agar media using the Streak Plate method. Bacterial colonies were inoculated into MRS agar media using a sterile ose by scraping into the media. Furthermore, MRS agar was incubated at 37ºC for 48 hours, anaerobically. The growing colonies were identified by gram staining.Identifikasi bakteri *Lactobacillus acidophilus* dengan pewarnaan gram
2) Bacteria were examined by Gram staining Gram staining was done by adding one drop of crystal violet to the preparation that had been smeared with 24-hour-old LAB isolate. The preparation was left for 1 minute and washed with distilled water. A total of 1 drop of iodine was added to the preparation, left for 2 minutes and rinsed with distilled water. The preparation was re-washed using 95% ethanol and rinsed under running water. LAB isolates were added safranin, rinsed under running water. The preparations were dried at room temperature, then observed under a microscope at 1000x magnification.
3) Isolation of bacteriocin produced by *Lactobacillus acidophilus*
Lactobacillus acidophilus bacterial isolates that grew on 48-hour-old MRS agar were inoculated into 10 ml of MRS broth media and incubated for 48 hours at 37ºC in an anaerobic atmosphere. Furthermore, the culture was centrifuged at 6000 rpm for 2x30 minutes. The liquid was taken as much as 5 mL and then put in effendof. The liquid was used as bacteriocin and ready for antibacterial testing.
4) Heating temperature treatment of bacteriocin 5 ml Bacteriocin was divided into 4 groups, each group was treated with heating temperature ranging from 40 ºC, 60 ºC, 80 ºC, and 100 ºC. Each treatment at this heating temperature was repeated twice. The heating treatment was carried out by putting bacteriocin into effendof with a volume of 1.25 mL for each treatment. After that, the bacteriocin was heated in a water bath with different temperatures for 30 minutes. Furthermore, the bacteriocin was tested for antibacterial against *Methicilin resistant Staphylococcus aureus* (MRSA) with the Kirby Bauer method.
5) *Methicilin resistant Staphylococcus aureus* (MRSA) test bacteria preparation
Inoculation of MRSA test bacteria in TSA media and incubation at 37°C for 1 x 24 hours in an aerobic atmosphere. Preparation of bacterial inoculum for MRSA testing was carried out by taking several MRSA colonies from TSA media using an ose and inserting them into 2 ml of BHI media. Turbidity correction was performed using 0.5% McFarland normalization standard equivalent to 1.5 × 108 CFU/mL.
6) Antibacterial testing of bacteriocins against *Methicilin resistant Staphylococcus aureus* (MRSA) by Kirby Bauer method MRSA test bacteria with a concentration of 1.5 × 108 CFU/mL were grown on Mueller Hinton Agar media by swabbing with a sterile cotton swab. Sterile cotton swabs were dipped into the test bacterial suspension evenly on the entire surface of the MHA media and then allowed to stand for 5-15 minutes so that the

suspension could seep into the media. Paper discs were dripped with 20 µl of bacteriocin supernatant suspension that had been given several heating temperature treatments. The discs were placed on MHA Media containing the test bacteria, then incubated at 37ºC for 24 hours in an aerobic atmosphere. The diameter of the inhibition zone formed was measured using a caliper with millimeter units. The diameter of the inhibition zone was used to determine the antimicrobial activity of *Lactobacillus acidophilus* bacteria against MRSA bacteria.

7) Data analysis
Inhibition zone diameter data from each treatment was tested using ANOVA test, to determine the effect of heating on bacteriocin activity produced by *Lactobacillus acidophilus* against MRSA.

RESULT AND DISCUSSION

(1) Result

The examination of bacteriocin activity produced by *Lactobacillus acidophilus* against MRSA was carried out by identifying *Lactobacillus acidophilus* and antibacterial tests. Identification by gram staining showed that *Lactobacillus acidophilus* bacteria are gram-positive with bacillus-shaped cells (Figure 1). On MRS agar media, the colonies are round and white-light brown in color.

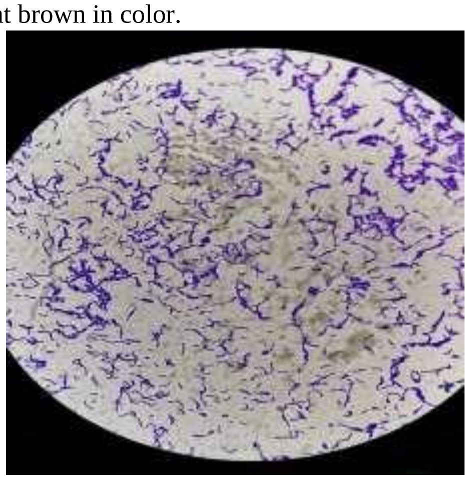


Figure 1. Bacterial cell shape of *Lactobacillus acidophilus* by Gram stain

Figure 2. showed the results of the examination of bacteriocin activity produced by *Lactobacillus acidophilus* against *Methicilin resistant Staphylococcus aureus* (MRSA) with various heating temperature treatments. The average diameter of the inhibition zone was 5.25 millimeters. This study shows that bacteriocin produced by *Lactobacillus acidophilus* bacteria has activity against MRSA bacteria.

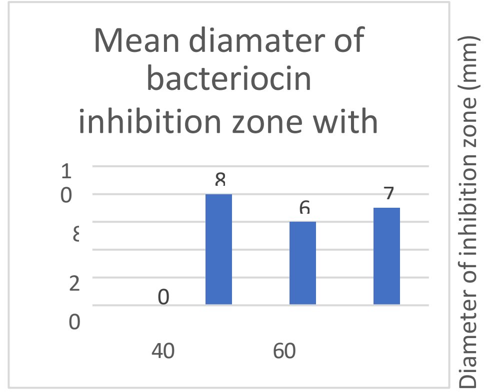


Figure 2. Diameter of inhibition zone of bacteriocin produced by *Lactobacillus acidophilus* against MRSA

The diameter of the bacteriocin inhibition zone with 60 ºC temperature treatment showed the highest antibacterial activity of 8 mm. At 40 ºC, bacteriocin has not shown its antibacterial activity. From these data, it is obtained that the optimal heating temperature for antibacterial activity of bacteriocin against MRSA is 60 ºC. ANOVA test analysis results obtained P = 0.072. The statistical test results prove that temperature does not affect the activity of Lactobacillus acidophilus bacteriocin against MRSA.

(2) Discussion

This study proves that bacteriocins produced by *L. acidophilus* have antibacterial power against *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria. temperature does not affect the activity of bacteriocins produced by *L. acidophilus* against MRSA. The optimal temperature of antibacterial activity of bacteriocin produced by *L. acidophilus* was 60°C with an average inhibition zone diameter of 8 mm.

The bacteriocin activity produced by *L.acidophilus* against MRSA is considered moderate. This is in accordance with the criteria of Ismail et al. (2017) that antibacterial inhibition zone activity can be grouped into four groups, namely weak activity (<5 mm), moderate (5-10 mm), strong (>10-20 mm) and very strong (>20-30 mm). The larger the clear zone on MHA media, the higher the level of antibacterial activity against pathogens. The antibacterial activity of bacteriocins produced by *L. acidophilus* isolate bacteria is included in the moderate category against *Methicillin-resistant Staphylococcus aureus* (MRSA). This study proves that the existence of temperature variations does not have a significant effect on the diameter of the inhibition zone produced by bacteriocins against the growth of *Methicillin-*

Doi: 10.18196/umygrace.v4i1.664

resistant Staphylococcus aureus (MRSA). The average value of the diameter of the inhibition zone formed by bacteriocin in all temperature treatments is 5.25 mm, the size of this diameter is included in the group of moderate antibacterial activity with a diameter range of 5-10 mm.

Heating temperature can affect protein stability and activity against all types of proteins (Wang et al., 2015). This effect is due to changes in protein structure at moderate heating temperatures (Ellouze et al., 2021). According to Widayati et al (2019), the antibacterial activity of bacteriocins against E. coli and S. aureus formed the highest inhibition zone at 100°C. Bacteriocins remain stable at heating temperatures, pH, and the administration of high concentrations of NaCl (Zanengeh., et al 2020).

Research proves that bacteriocins remain stable at high, normal, and low temperatures as seen from the size of the diameter of the inhibition zone on the growth of pathogenic bacteria (Sukmawati et al., 2022). Although bacteriocins are resistant to high temperatures, research shows that there is a 25% decrease in the antibacterial ability of nisin-type bacteriocins when heating at 160°C for a duration of 5 minutes and a 60% decrease when heating at 180°C for a duration of 5 minutes (Holcapkova et al., 2018). Besides being influenced by pH and temperature, the antibacterial activity of bacteriocins is also influenced by the length of incubation time. Research proves that the highest activity of bacteriocins against pathogenic bacteria is in lactic acid bacteria with an incubation time of 27°C for 14 hours at pH 6 (Marwati et al., 2018).

The results of this study are similar to research by Zanengeh et al. (2020) that the activity of antibacterial bacteriocins is stable to different temperature treatments. However, the results of this study differ from research conducted by Widayati et al. (2019) which showed that the highest antibacterial activity of bacteriocins against Escherichia coli and Staphylococcus aureus was produced by bacteriocins with heating at 100°C.

Bacteriocins are a number of antibacterial peptides known as bacteriostatic and bactericidal against bacterial growth. Bacteriocins are synthesized in ribosomes that undergo modification after the translational process and then converted into an active form (Mokoena, 2017).

The growth of pathogenic bacteria associated with specific proteins contained in lactic acid bacteria genes can be inhibited by bacteriocins (Hols et al., 2019). Bacteriocins obtained from gram-positive bacteria are able to work by damaging the integrity of the bacterial cell membrane. There is one type of antibacterial bacteriocin that forms pores in the bacterial cell membrane and after that there will be an increase in the permeability of the cell which results in bacterial death. Other types of bacteriocins also produce antibacterial properties by inhibiting enzymes during the peptidoglycan biosynthesis process. The accumulation of peptidoglycan in the cytoplasm as a result of this enzyme inhibition will then result in disruption of the bacterial cell membrane (Simons et al., 2020).

Figure 3. shows the mechanism of how bacteriocins work in killing bacteria

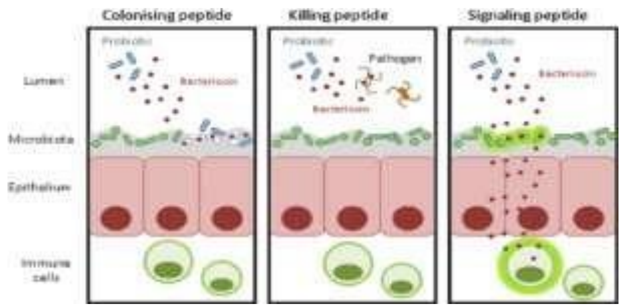


Figure 3. Bacteriocin mechanism kills pathogen

In a study, Yao Wang (2019) conducted a study against Listeria monocytogenes using bacteriocins from Lactobacillus plantarum LPL-1 isolated from animals. The results suggest that bacteriocins can stop the growth of L. monocytogenes by making the cell membranes of the L. Monocytogens more acidic and creating pores inside the bacterial membrane. In another study, Sam Woong Kim (2020) and colleagues assessed the effect of bacteriocins produced by *Lactobacillus taiwanensis* against *Salmonella gallinarum* and *Escherichia coli*. Resultantly, it was observed that the bacteriocin produced by *L. taiwanensis* could inhibit bacterial growth through the lysis of the membrane of pathogenic bacteria and thus damage their protein structure. Based on the results of the mentioned studies, bacteriocins can be used as a tool to inhibit the bacteria that cause food spoilage.

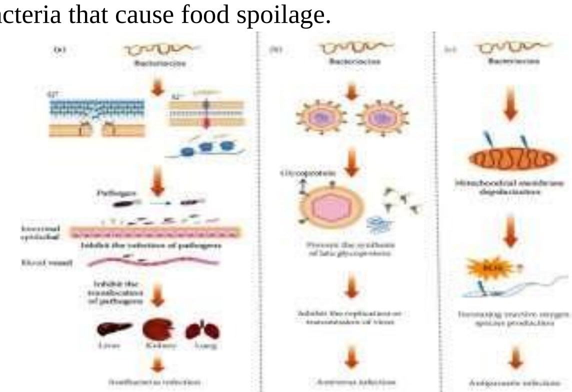


Figure 4. shows the inhibitory activity against pathogens

Bacteriocins fight various pathogenic microorganisms through various mechanisms, protecting the human body from infection. (a) Bacteriocins have the ability to kill pathogenic bacteria directly by stopping the biosynthesis of bacterial cell walls by complexing lipid II and forming pores on the cell membrane. In addition, bacteriocins can interfere with the sensing of the bacterial population as signaling molecules, or enter the cell through transporters and interact with very important enzymes, such as ATP-dependent proteases. This eliminates the presence of pathogenic bacteria in the body and reduces the tendency of bacteria to enter the lungs, kidneys, and liver, among others. (b) For viruses, bacteriocins can prevent the proliferation or transfer of viruses by preventing the production of glycoproteins in the late stage of virus replication. (c) For parasites, bacteriocins can prevent parasites by depolarizing the mitochondrial membrane and the production of reactive oxygen species.

CONCLUSION AND RECOMMENDATION

This study showed that bacteriocin produced by *L. acidophilus* has antibacterial activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria. The treatment of various heating temperatures did not affect the antibacterial activity of bacteriocins produced by *L.acidophilus* against MRSA. The optimal temperature to obtain antibacterial activity of bacteriocins produced by *L.acidophilus* was 60ºC, with an inhibition zone diameter of 8 mm. The presence of antibacterial activity of bacteriocins can be developed further research, to obtain complete information about the utilization of bacteriocins as antibacterial against pathogenic bacteria, especially those that are resistant to various antibiotic substances.

REFERENCE

1. Abbott I., Jenney A., Jeremiah C., Mirèeta M., Kandiah J., Holt D., dkk. (2015). Mengurangi aktivitas ceftaroline in-vitro dengan melakukan pengujian pada strain klinis Staphylococcus aureus (MRSA) yang resisten terhadap metisilin CC239 dari Australia. Antimikroba. Agen Kemoterapi. 59 7837–7841
2. Almira Disya Salsabil, Maftuchah Rochmanti, Agung Dwi Wahyu Widodo. (2022). Vancomycin Monotherapy vs Alternative Antibiotics for MRSA Patients: A Systematic Review. International Journal of Research Publications, Volume: 92, Issue: 1.
3. Anggraini Pujasari. Pengaruh jenis bakteri asam laktat dan konsentrasi inokulum terhadap produksi asam laktat dari air kelapa. [Malang]: Universitas Islam Negeri Maulana Malik Ibrahim.; 2019. bacteria and yeast. Food Res Int. 2015;76(Pt. 3):787–95.
4. Ellouze, M., Vial, C., Attia, H., & Ayadi, M. A. (2021). Effect of pH and heat treatment on structure, surface characteristics and emulsifying properties of purified camel β-casein. Food Chemistry, 365, 130421. https://doi.org/10.1016/j.foodchem.2021.13042
5. Erlin, E. et al. (2020) 'Deteksi Methicilin Resistant Staphylococcus aureus (MRSA) Sebagai Penyebab Infeksi Nosokomial Pada Alat-Alat di Ruang Perawatan Bedah', Quagga: Jurnal Pendidikan dan Biologi. Doi:10.25134/quagga. v12i2.2671. Freire AL, Ramos CL, Schwan RF. Microbiological and chemical parameters during cassava based- substrate fermentation using potential starter cultures of lactic acid
6. Heilbronner, S., Krismer, B., Brö tz-Oesterhelt, H., & Peschel, A. (2021). The microbiome- shaping roles of bacteriocins. Nature Reviews. Microbiology, 19(11), 726–739. https://doi.org/10.1038/S41579-021-00569-W
7. Hernandez-Gonzalez, J. C., Martinez-Tapia, A., Lazcano-Hé rnandez, G., Garcia-Perez, B. E., & Castrejon- ´Jiménez, N. S. (2021). Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials, Probiotics, and Immunomodulators in Veterinary Medicine. Animals: An Open Access Journal from MDPI, 11(4). https://doi.org/10.3390/ANI11040979
8. Holcapkova, P., Hurajova, A., Bazant, P., Pummerova, M., & Sedlarik, V. (2018). Thermal stability of bacteriocin nisin in polylactide-based films. Polymer Degradation and Stability, 158, 31–39. https://doi.org/10.1016/j.polymdegradstab.2018.10.01 9
9. Hols, P., Ledesma-Garcia, L., Gabant, P., & Mignolet, J. (2019). Mobilization of Microbiota Commensals ´ and Their Bacteriocins for Therapeutics. Trends in Microbiology, 27(8), 690–702. https://doi.org/10.1016/J.TIM.2019.03.007
10. Hu, C.H.; Ren, L.Q.; Zhou, Y.; Ye, B.C. Characterization of antimicrobial activity of three Lactobacillus plantarum strains isolated from Chinese traditional dairy food. *Food science & nutrition* **2019**, *7*, 1997-2005, https://doi.org/10.1002/fsn3.1025.
11. Ismail, Y. S., Yulvizar, C., & Putriani, P. (2017). Isolasi, Karakterisasi, dan Uji Aktivitas Antimikroba Bakteri Asam Laktat dari Fermentasi Biji Kakao (Theobroma cacao L.). Jurnal Bioleuser, 1(2). https://jurnal.usk.ac.id/bioleuser/article/view/9072
12. Kim, S.W.; Ha, Y.J.; Bang, K.H.; Lee, S.; Yeo, J.-H.; Yang, H.-S.; Kim, T.-W.; Lee, K.P.; Bang, W.Y. Potential of bacteriocins from Lactobacillus taiwanensis for producing bacterial ghosts as a next generation vaccine. *Toxins* **2020**

13. Marwati, T., Cahyaningrum, N., Widodo, S., Januarsyah, T., & Purwoko. (2018). Inhibitory activity of bacteriocin produced from Lactobacillus SCG 1223 toward L. monocytogenes, S. thypimurium and E. coli. IOP Conference Series: Earth and Environmental Science, 102, 012091. https://doi.org/10.1088/1755- 1315/102/1/012091
14. Mokoena, M. P. (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules (Basel, Switzerland), 22(8). https://doi.org/10.3390/MOLECULES22081255
15. Negash, A. W., & Tsehai, B. A. (2020). Current Applications of Bacteriocin. *International Journal of Microbiology*, *2020*, 1–7. https://doi.org/10.1155/2020/4374891
16. Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. *Microb Cell Fact*. 2014;13(Suppl 1): S3.
17. Rahmadi A. Bakteri asam laktat dan mandai cempedak. Mulawarman University Press. Samarinda: Mulawarman University Press; 2019.
18. Roces C, Rodríguez A, Martínez B. Bakteriosin aktif dinding sel dan penerapannya di luar aktivitas antibiotik . Protein Antimikroba Probiot . 2012; 4 (4):259-272
19. Simons, A., Alhanout, K., & Duval, R. E. (2020). Bacteriocins, Antimicrobial Peptides from Bacterial Origin: Overview of Their Biology and Their Impact against Multidrug-Resistant Bacteria. Microorganisms, 8(5), 639. https://doi.org/10.3390/microorganisms8050639
25.
20. Suganya, K., Murugan, T., & Murugan, M. (2013). Isolation and characterization of probiotic lactic acid bacteria from milk and curd samples. International Journal of Pharma and Bio Sciences, 4(1): (B) 317-324.
21. Sukmawati, S., Sipriyadi, S., Yunita, M., Dewi, N. K., & Noya, E. D. (2022). Analysis of bacteriocins of lactic acid bacteria isolated from fermentation of rebon shrimp (Acetes sp.) in South Sorong, Indonesia as antibacterial agents. Biodiversitas Journal of Biological Diversity, 23(7). https://doi.org/10.13057/biodiv/d230763
22. Wang, H., She, Y., Chu, C., Liu, H., Jiang, S., Sun, M., & Jiang, S. (2015). Preparation, antimicrobial and release behaviors of nisin-poly (vinyl alcohol)/wheat gluten/ZrO2 nanofibrous membranes. Journal of Materials Science, 50(14), 5068–5078. https://doi.org/10.1007/s10853-015-9059-0
23. Widayati, O., Bachruddin, Z., Hanim, C., Yusiati, L. M., & Umami, N. (2019). Bacteriocin Activity of Lactic Acid Bacteria Isolated from Rumen Fluid of Thin Tail Sheep. Buletin Peternakan, 43(3). https://doi.org/10.21059/buletinpeternak.v43i3.36837
24. Zangeneh, M., Khorrami, S., & Khaleghi, M. (2020). Bacteriostatic activity and partial characterization of the bacteriocin produced by L. plantarum sp. isolated from traditional sourdough. Food Science & Nutrition, 8(11), 6023–6030. https://doi.org/10.1002/fsn3.1890

Doi: 10.18196/umygrace.v4i1.666

Optimization of Temperature to Bacteriocin by *Lactobacillus plantarum* Against *Methicillin-Resistant Staphylococcus aureus*

***Azizah* Balqis Salsabilla[1, a], and *Lilis* Suryani[2, b]**
[1]Medicine Studies, Faculty of Medicine and Health Science, University of Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183
[2]Department of Microbiology, Faculty of Medicine and Health Science, University of Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183

Email: azizah.balqis.fkik22@mail.umy.ac.id[a]; lilis.suryani@umy.ac.id[b]

ABSTRACT

Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogenic bacterium that is resistant to antibiotics. MRSA causes skin and soft tissues, lungs, blood flow, endocarditis, and osteomyelitis. The rate of MRSA infection in the world is 14.69% and in Indonesia by 25%-65%. They have resistance to beta-lactam antibiotics, penicillin, cephalosporin, and tetracycline. Bacteriosin is produced by lactic acid bacteria. The bacteriosins produced by Lactobacillus Plantarum has the ability to inhibit the growth of positive gram bacteria. **Purpose:** The study aims to knwoing how the temperature affects the bacteriologic activity produced by Lactobacillus plantarum affects the MRSA. Methode: The study employed quasi-experimental laboratory methods. Lactobacillus plantarum is grown on De Man Rogosa and Sharpe (MRS) Liquid media for 48 hours. Bacteria at 6000 RPM can be traced to a bacterium through a bacterium. The fluid formed was tested by its antibacterial effect on MRSA bacteria using the Kirby Bauer method. Bacteria are treated with different temperatures; 40°C, 60°C, 80°C, and 100 °C. The diameter of the obstructive zone formed during the testing process is measured by a millimeter. The data is analyzed by ANOVA methods. **Finding:** The bacteria generated by Lactobacillus plantarum on the MRSA showed an average diameter of the inhibition zone of 5.63 mm. The spectrum of the largest antibacterial activity indicates that the bacteria is heated at 60 °C temperature. **Conclusion:** The study showed results that the temperature did not affect the bacteria's antibacterial activity that the Lactobacillus plantarum produced on the MRSA. **Originality/Implication:** The results of this study prove that variations in temperature treatment do not affect the bacteria generated by the Lactobacillus plantarum in impeding or killing MRSA.

ABSTRAK

Pendahuluan: Staphylococcus aureus (MRSA) yang resisten terhadap Methicillin-resistant adalah bakteri patogen yang resisten terhadap antibiotik. MRSA menyebabkan kulit dan jaringan lunak, paru-paru, aliran darah, endokarditis, dan osteomielitis. Tingkat infeksi MRSA di dunia adalah 14,69% dan di Indonesia sebesar 25%-65%. Mereka memiliki resistensi terhadap antibiotik beta-laktam, penisilin, sefalosporin, dan tetrasiklin. Bakteriosin diproduksi oleh bakteri asam laktat. Bakteriosin yang diproduksi oleh Lactobacillus Plantarum memiliki kemampuan untuk menghambat pertumbuhan bakteri gram positif. **Tujuan:** Penelitian ini bertujuan untuk mengetahui bagaimana suhu mempengaruhi aktivitas bakteriologis yang diproduksi oleh Lactobacillus plantarum mempengaruhi MRSA. **Metode:** Studi ini menggunakan metode laboratorium kuasi-eksperimental. Lactobacillus plantarum ditanam di media Cair De Man Rogosa dan Sharpe (MRS) selama 48 jam. Bakteri pada 6000 RPM dapat ditelusuri ke bakteri melalui bakteri. Cairan yang terbentuk diuji dengan efek antibakterinya pada bakteri MRSA menggunakan metode Kirby Bauer. Bakteri diperlakukan dengan suhu yang berbeda; 40°C, 60°C, 80°C, dan 100 °C. Diameter zona obstruktif yang terbentuk selama proses pengujian diukur dengan satu milimeter. Data dianalisis dengan metode ANOVA. **Hasil:** Bakteri yang dihasilkan oleh Lactobacillus plantarum pada MRSA menunjukkan diameter rata-rata zona penghambatan 5,63 mm. Spektrum aktivitas antibakteri terbesar menunjukkan bahwa bakteri dipanaskan pada suhu 60 °C. **Kesimpulan:** Studi ini menunjukkan hasil bahwa suhu tidak mempengaruhi aktivitas antibakteri bakteri yang diproduksi Lactobacillus plantarum pada MRSA. **Orisinalitas/Implikasi:** Hasil penelitian ini membuktikan bahwa variasi dalam perlakuan suhu tidak mempengaruhi bakteri yang dihasilkan oleh Lactobacillus plantarum dalam menghambat atau membunuh MRSA.

Doi: 10.18196/umygrace.v4i1.666

INTRODUCTION

Methicillin-Resistant Staphylococcus aureus is a collection of bacteria produced from *S.aureus* cultures that are resistant or resistant to antibiotics such as must-have and beta-lactam groups. The MRSA resistance process is formed due to the Penicilin Binding Protein (PBP2a) produced by *Staphylococcus aureus* which is regulated by the mecA gene which has a low affinity for methicillin (indas W. *et al.*, 2023). *Staphylococcus aureus* Is a gram-positive bacterium that has the enzyme catalase, is cocci-shaped, and is an opportunistic pathogen in humans. MRSA can result in death, increased pain rates, and increased nosocomial infections. (Yulianto A. *et al.*, 2019)

Peptida atau protein yang disintesis dalam ribosom oleh bakteri asam laktat disebut bakteriosin. Bakteriosin memiliki kemampuan bakterisida yang terkait erat dengan bakteri penghasilnya, terutama bakteri gram positif. Sebagai antibakteri ada beberapa mekanisme utama bakteriosin, yaitu degradasi sel DNA, pembentukan pori, dan menghentikan sintesis peptidoglikan. (Merliana P. *et al.*, 2020)

In the Al-Qur'an, Allah says in the letter Ash-Syu'ara verse 80 "And when I am sick, He is the only one who heals me from illness, there is no healer for me other than Him."

Various strains of lactic acid bacteria with various types of closely related foods can produce bacteriosin so that it effectively kills pathogens in food such as *Staphylococcus aureus*, *Shigella flexneri, Escherichia colli, clostridium botulinum, Pseudomonas fluorescens,* and other. Bacteriocin has high stability, has the potential for synergistic effects, and has no side effects. In the food industry it is very important to choose the appropriate antibiotic-producing strain. (Atieh D. *Et al.*, 2022)

Lactobacillus plantarum Is a lactic acid bacterium that produces secondary metabolites in the form of bacteriosin used in the health world for antimicrobials and food products. The bacteriosin produced by *L.plantarum* has the ability to be antimicrobial at 24 to 36 hours. The 28th hour is the optimum production time of bacteriosin and has the highest antibacterial activity (Nadia P. *et al.*, 2019). *Lactobacillus plantarum* Grows at dawn if it is at a temperature between 28 and 45 °C, while it will grow optimally at 37°C. (Neelja S. *Et al.*, 2021)

So far there is still little, and no research has even been found on the effect of warming temperature on the bacteriosin activity produced by *Lactobacillus plantarum* on MRSA. This research was conducted to determine the influence of temperature on the activity of bacteriosin produced by *Lactobacillus plantarum* against MRSA and obtain the optimum temperature of bacteriosin activity to kill MRSA bacteria.

LITERATURE REVIEW

(1) *Methicillin resistant Staphylococcus aureus* (MRSA)

MRSA is a bacterium that arises due to resistance to antibiotics. The spread of this bacterium is often found in health care centers through contact between patients and medical devices or medical personnel. (Santy pristianingrum et al., 2021)

MRSA is 100% resistant to antibiotics such as cefotaxim and ceftriaxone. 80% resistant to azithromycin, clindamycin, and erythromycin. 60% resistant to ofloxacin. Also, 40% were resistant to ciprofloxacin. Meanwhile, antibiotics that are 100% sensitive to MRSA are trimethoprim-sulfamethoxazole, chloramphenicol, and gentamycin. (Definov T. Et al., 2014)

MRSA has a correlation to malignancy. Most occur in incoming solid tumors such as neck and head tumors, lung tumors, and buli tumors. (Moehammad I. Et al., 2014)

(2) *Lactobacillus plantarum*

Lactobacillus plantarum is a homofermentative lactic acid bacteria that is able to convert carbohydrates into lactic acid. *L.plantarum* has a bactericidal effect and can inhibit the growth of bacteria from lactic acid associated with decreasing the pH of the environment to 3-4.5. Where generally patoge will grow at a pH of about 6-8. (Ririn P. Et al., 2011)

L.plantarum is a gram-positive bacterium. Has a size of 0.9-1.2 to 1.0-8.0 in the form of a rod, can be single or chain. (Akhmad H. Et al., 2019)

The original habitat of *L.plantarum* is the mucous membrane of animals or humans, waste, plants, fermented foods such as sour milk, sour dough, and many more. (Bambang P., et al 2016)

(3) Bacteriocins

Bakterioscins is a protein produced by a bacterium to inhibit the growth of other bacteria. (Sri M. et al., 2022)

Bacteriosin can be used as a natural preservative to prevent the growth of bacteria also called biopreservative agents. (Nurraifah et al., 2021)

METHOD

(1) Study design

This research is a laboratory experiment, the research was carried out in May - June 2024 and is located in the Microbiology laboratory of the Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta. This research has received permission from the FKIK UMY HEALTH

Doi: 10.18196/umygrace.v4i1.666

RESEARCH ETHICS COMMITTEE no. 051/EC-EXEM-KEPK FKIK UMY/IV/2022.

(2) Samples

The research sample is Lactobacillus plantarum collection from PAU UGM laboratory on Jl. Teknika Utara, Caturtunggal, Depok, Sleman Regency, Special Region of Yogyakarta. The test bacteria used is Methicillin resistant Staphylococcus aureus (MRSA) local isolate from the FK UGM Microbiology Laboratory.

(3) Tools and Materials

The tools used in this study include Petri cup (Iwaki pyrex), Ose needle, object glass, test tube, erlemeyer tube (Iwaki pyrex), beaker glass (Iwaki pyrex), autoclave, microscope, incubator, Biological Safety Cabinet, bunsen, refrigerator, centrifugation, water bath and caliper.

Ingredients used in this study include Lactobacillus plantarum isolate, De Man Rogosa and Sharpe (MRS) agar media, De Man Rogosa and Sharpe (MRS) broth, aquades, violet crystals, iodine, 95% ethanol, safranin, dapar phosphate, NaOH, HCl, Mueller Hinton Agar (MHA), Trypticase Soy Agar (TSA)

(4) Research Prosedure

Rejuvenation of Lactobacillus plantarum bacterial culture

Lactobacillus plantarum is carried out by subculture on MRS media so that by using the Streak Plate method. Colonies of bacteria are inoculated into MRS media in order to use sterile ose by being scratched into the media. Then MRS should be incubated at a temperature of 37oC for 48 hours, anaerobically. The growing colony is identified by gram staining.

Identify Lactobacillus plantarum bacteria by gram staining

Bacteria are checked with Gram staining. Gram coloring is done by adding one drop of violet crystals to the preparation that has been smeared with BAL isolate which is 24 hours old. The preparation is left for 1 minute and washed with aquades. A total of 1 drop of iodine is added to the preparation, left for 2 minutes and rinsed with aquades. The preparation is re-washed using 95% ethanol and rinsed in running water. BAL isolate added safranin, rinsed in running water. The preparation is dried at room temperature, then observed under a microscope with a magnification of 1000x.

Isolation of bacteriosin produced by Lactobacillus plantarum

Lactobacillus plantarum bacterial isolate that grows on MRS to be 48 hours old, is inoculated into 10 ml of MRS broth media then incubated for 48 hours at 37oC in an anaerobic atmosphere. Next the culture is centrifugated at a speed of 6000 rpm for 2x30 minutes. The liquid is taken as much as 5 mL and then inserted into the effendof. The liquid is used as bacteriosin and is ready for antibacterial tests.

Heating temperature treatment of bacteriosin

5 ml of Bacteriosin is divided into 4 groups, each group is treated with a heating temperature ranging from 40 oC, 60 oC, 80 oC, and 100 oC. Each treatment at this heating temperature is done 2 repetitions. The heating treatment is carried out by inserting bacteriosin into the effendof with a volume of 1.25 mL of each treatment. After that bacteriosin is heated in a water bath with different temperatures for 30 minutes. Furthermore, bacteriosin was tested for antibacterial against Methicilin resistant Staphylococcus aureus (MRSA) by the Kirby Bauer method.

Test bacterial preparation Methicilin resistant Staphylococcus aureus (MRSA)

MRSA test bacterial inoculation in TSA media and incubation at 37°C for 1 x 24 hours in aerobic atmosphere. Preparation of bacterial inoculum for MRSA testing was performed by taking several MRSA colonies from TSA media using ose and inserting them into 2 ml of BHI media. Turbidity correction was performed using the McFarland 0.5% normalization standard equivalent to 1.5 × 108 CFU/mL.

Bacteriosin antibacterial testing against Methicilin resistant Staphylococcus aureus (MRSA) by Kirby Bauer method

MRSA test bacteria with a concentration of 1.5 × 108 CFU/mL are planted on Mueller Hinton Agar media by swab using a sterile cotton stick. Sterile cotton sticks are dipped in the test bacterial suspension evenly on the entire surface of the MHA media then allowed to stand for 5-15 minutes so that the suspension can soak into the media. The paper disc was treated with a 20 μl of bacteriosin supernate suspension that had been given some heating temperature treatment. The disc is placed on

Doi: 10.18196/umygrace.v4i1.666

top of the MHA Media containing the test bacteria, then incubated at 37oC for 24 hours in an aerobic atmosphere. The diameter of the barrier zone formed is measured using a caliper with millimeter units. The diameter of the barrier zone is used to determine the antimicrobial activity of Lactobacillus plantarum bacteria against MRSA bacteria.

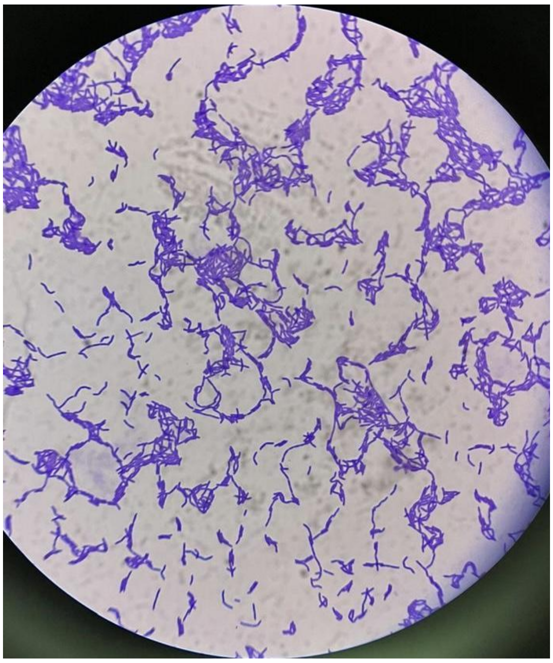
(5) Data analysis

The resistance zone diameter data of each treatment were tested using the ANOVA test, to determine the effect of warming on the bacteriosin activity produced by Lactobacillus acidophilus on MRSA.

RESULT AND DISCUSSION

(1) Result

Examination of bacteriosin activity produced by Lactobacillus plantarum against MRSA is carried out by identification of Lactobacillus plantarum and antibacterial test. Identification by gram staining shows that Lactobacillus plantarum bacteria are gram positive with bacillus-shaped cells (Figure 1). In MRS media so that the colony is round and light white brown in color.



Firuge 1. Bacterial cell form of Lactobacillus plantarum with Gram staining

Table 1. The difference in temperature-based inhibition zones

Mean diameter of bacteriocin inhibition zone with various temperature treatments				
temperature	40 oC	60 oC	80 oC	100 oC
diameter of inhibition	0 mm	8 mm	7 mm	7.5 mm

Table 1. Showing the results of examination of bacteriosin activity produced by Lactobacillus plantarum against MRSA with various heating temperature treatments. The average barrier zone diameter is 5,625 millimeters. This research shows that bacteriosin bacteria produced by Lactobacillus plantarum bacteria have activity against MRSA bacteria.

The diameter of the bacteriosin inhibition zone with a temperature treatment of 60 oC shows the highest antibacterial activity of 8 mm. At a temperature of 40 oC bacteriosin has not shown its antibacterial activity. From the data, information was obtained that the optimum heating temperature for antibacterial activity bakteriosin terhadap MRSA sebesar 60 oC. Hasil analisis uji ANOVA diperoleh P=0.069. Dari hasil uji statistik tersebut membuktikan bahwa suhu tidak mempengaruhi aktivitas bakteriosin *Lactobacillus plantarum* terhadap MRSA.

(2) Discussion

This research proves that the bacteriocin produced by *L.plantarum* has antibacterial power againstMRSA bacteria. Temperature does not affect the bacteriocin activity produced by *L.plantarum* against MRSA. The optimum temperature of bacteriocin antibacterial activity produced by *L.plantarum* is 60 ºC with an average barrier zone of 8 mm. The bacteriocin activity produced by *L.plantarum* against MRSA is moderate. There are 4 categories of antimicrobial resistance zone activity groups, namely: very strong activity (>20-30mm), strong (> 10-20 mm), medium (5-10 mm), and weak (<5 mm). The diameter of this resistance zone is produced from the measurement of the clear zone produced by the paper disc and measured using mm units. (Frans U. D. *Et al.*, 2019)

Inhibition of *Staphylococcus aureus* and *Escherichia coli* is capable of being carried out by bacteriocin produced by *Lactobacillus plantarum* obtained by dialysis and purification stages. At room temperature up to 100 °C bacteriocin *Lactobacillus plantarum* will remain stable with a weak category at 100°C, a moderate category at at 80°C, a strong category at a temperature of 65°C. Testing is done on *Escherichia coli* bacteria. The bacteriosin produced by *Lactobacillus plantarum* is very sensitive to the enzyme's pepsinogen and trypsin and with a decrease in protein concentration by 78.18% and 67.56% (Nadzifah & Mafrudhotin, 2022). This is in line with this study that the most optimal temperature is in the range of 60-65°C.

Doi: 10.18196/umygrace.v4i1.666

Generally, the antibacterial mechanism of Bacteriosin *Lactobacillus* is to damage the cell membrane of the target bacteria by forming pores to affect the permeability of the cell membrane (Allysa S. *Et al*., 2022). Inhibition of the growth of *Escherichia coli* bacteria, *Staphylococcus aureus*. And *Salmonella typhi* can be done by the bacteriosin bacterium *Lactobacillus plantarum* (Rafika S. *Et al.*, 2016)

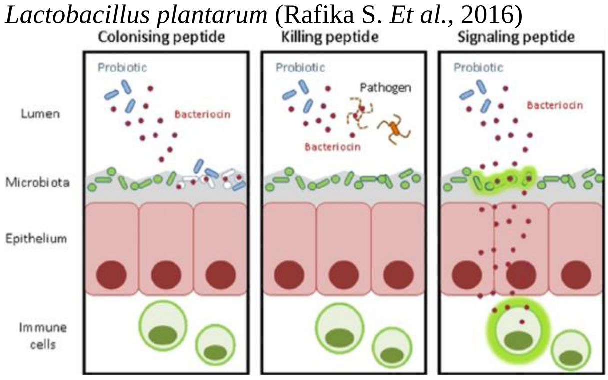


Figure 2. Shows the mechanism of how bacteriosin works in killing bacteria.

There is also bacteriosin 13B which comes from the isolation of the cow colon. This bacteriosin is thought to have a molecular weight of less than 10 kDa and has antimicrobial activity against bacteria *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* (Ni P. *et al.*, 2017). Glucose is the best source of carbon in influencing bacteriosin activity (Kusmiati & Amarila M. 2002). Inhibition to cell wall synthesis, nucleic acid synthesis and major metabolism, and protein synthesis are the working mechanisms of bacteriosin (Sri Mastuti, 2022).

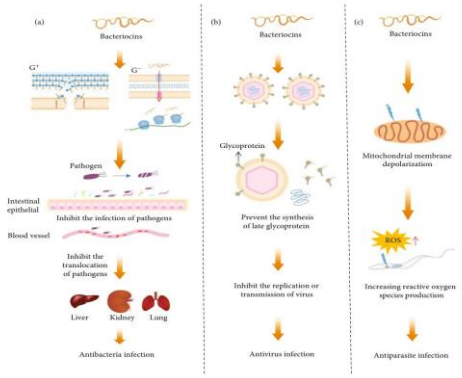


Figure 3. Show inhibitory activity against pathogens

To inhibit various pathogenic microorganisms that attack the body, bacteriosin has several mechanisms. (a) If due to bacteria, bacteria are killed by bacteriosin by inhibiting the biosynthesis of bacterial cell walls by complexing lipid II and forming pores in the cell membrane. On the other hand, a bacterial sensing system that is useful as a signaling molecule can also be disturbed by bacteriosin through Transporters and interact with protease enzymes that need ATP. Thus, pathogenic bacteria do not easily enter the lungs, kidneys, and liver. (b) resistance to the virus, bacteriosin prevents the production of glycoproteins in the virus and prevents its proliferation. (c) while for parasites, bacteriosin through mitochondrial membrane depolarization and the production of reactive oxygen species will prevent parasite attack. (Fuqing Huang et al, 2021)

There are other studies that methanol extract has the ability to inhibit the growth of MRSA bacteria (Irma S. & Risa N., 2017). In other studies, it is also mentioned that guava stem methanol extract has resistance strength equivalent to linezolid antibiotic resistance (Risa N. & Yunita., 2012). The isolation carried out on Holothuria scabra also has the potential to be used as an anti-MRSA agent because it has a fairly large resistance zone [19]. Garlic and turmeric are medicinal plants that can be used to inhibit the growth of MRSA bacteria (Dwi H. *et al*., 2010)

COCLUSION AND RECOMMENDATION

The results of this study show that there is no influence between temperature and bacteriosin activity produced by *Lactobacillus plantarum* on MRSA. The clear zone produced by bacteriosin at a temperature of 40°C, 60°C, 80°C, 100°C has an average diameter of 5,625 mm. Bacteriosin has the largest resistance zone at a temperature of 60°C with a diameter of 8 mm. Further research needs to be done with more temperature variations and may use bacteriosin produced by other bacteria

REFERENCES

1. Indas, W. R., Nurfitri, A., Rafika, R., & Joyce, V. T. (2023). Deteksi Bakteri MRSA *Methicillin-Resistant Staphylococcus aureus* pada Sampel Darah Pasien Rawat Inap. Jurnal Ilmu Alam dan Lingkungan, 14 (1), 48-54. https://journal.unhas.ac.id/index.php/jai2/article/view/26212/9562

2. Yulianto A., Khoirun N., A'yunil H., & Elvina D. I. (2019). Aktivitas Nanokomposit ZNO-Ag dalam Menghambat Pertumbuhan Bakteri *Methicillin Resistant Staphylococus aureus* (MRSA). PROSIDING. (Vol. 1, No. 1) https://conference.upgris.ac.id/index.php/snse/article/view/189

3. Merliana P. F., Imam M., & Trianing T. K. A. (2020). Pemanfaatan Bakteriosin dari Produk Fermentasi sebagai Antibakteri terhadap *Staphylococcus aureus.* Indonesia Medicus Veterinus, 9 (9), 835-848. https://xjournals.com/collections/articles/Artic

Doi: 10.18196/umygrace.v4i1.666

le?qt=pNGIy7BUHXUapgsdP1tZPyK/D+1y+a4ahNz0hlOJyxucp965YWCkwsoJhvS/x9De

4. Atieh D., Arezoo A., Marzieh M. A., Elnaz O., Malihe T., Masoume H. Z., & Amir D. E. (2022). Bacteriosins: Properties and Potential use as antimicrobials. *Journal of Clinical Laboratory Analysis,* 36 (1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8761470/

5. Nadia P., Rafika S., & Pratiwi A. (2019). Penentuan Waktu Optimum Produksi Bakteriosin dari *Lactobacillus plantarum* Terhadap Bakteri Patogen *Staphylococcus aureus* dan *Escherichia coli*. (Vol 4. No. 1). https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/37710/75676584136

6. Neelja S., Nambram S. S., Shilpa M., Manish K., & Jugsharan S. V. (2021). Rhizospheric *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) strain exhibit bile salt hydrolysis, hypocholestrolemic and probiotic cpabilities in vitro. National Library of Medicine. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8316525/
7. Santy P., Baiq L. Z., Zainul M., Fergy D. P., & Rolly A. (2021). Deteksi Methicillin Resistant Staphylococcus aureus (MRSA) Pada Peralatan Medis yang Digunakan di Ruang Rawat Inap RSUD Provinsi NTB. Jurnal Analis Medika Biosains (JAMBS), (Vol. 8, No. 1). http://jambs.poltekkes-mataram.ac.id/index.php/home/article/view/220/163
8. Definov T., Rita E., & Ligat P. (2014). Identifikasi dan Resistensi Bakteri Methicillin Resistant Staphylococcus aureus (MRSA) dari ulkus diabetikum derajat I dan II wegner di bagian penyakit dalam RSUD Arifin Ahmad. Jurnal Online Mahasiswa UNRI, (Vol. 1, No. 2). https://jom.unri.ac.id/index.php/JOMFDOK/article/view/2852/2767
9. Mochammad I., Suhendro S., Tonny L., & Murdani A. (2014). Faktor Risiko Methicillin Resistant Staphylococcus aureus pada Pasien Infeksi Kulit dan Jaringan Lunak di Ruang Rawat Inap. Jurnal Penyakit Dalam indonesia, 1 (1), 3-14. https://scholarhub.ui.ac.id/cgi/viewcontent.cgi?article=1404&context=jpdi
10. Ririn P., Putranti A., & Gina A. (2011). Aktivitas Metabolit Bakteri Lactobacillus plantarum dan Perannya dalam Menjaga Kesehatan Saluran Pencernaan. Konferensi Nasional Sains dan Aplikasinya. https://www.researchgate.net/profile/Ririn-Puspadewi/publication/282250708_Aktivitas_Metabolit_Bakteri_Lactobacillus_plantarum_dan_Perannya_dalam_Menjaga_Kesehatan_Saluran_Pencernaan/links/560969b608ae840a08d3a923/Aktivitas-Metabolit-Bakteri-Lactobacillus-plantarum-dan-Perannya-dalam-Menjaga-Kesehatan-Saluran-Pencernaan.pdf?origin=publication_detail&_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uRG93bmxvYWQiLCJwYWdlIjoicHVibGljYXRpb25Eb3dubG9hZCIsInByZXZpb3VzUGFnZSI6InB1YmxpY2F0aW9uIn19
11. Akhmad H., Jajang G., & Ellin H. (2019). Potensi Semyawa Metabolit yang Dihasilkan Lctobacillus plantarum Sebagai Bahan Biopreservasi dan Anti Bakteri pada Bahan Pangan Asal Hewan. JITP, (Vol. 7, No. 2). https://journal.unhas.ac.id/index.php/peternakan/article/download/6811/4590
12. Bambang P. & Titik B. (2016). Prevalensi dan Diversitas Lactobacillus sp. pada Susu Kambing Etawa Segar. Seminar nasional hasil penelitian dan pengabdian masyarakat, 57-60. https://sipora.polije.ac.id/20519/1/24.0%20%28artikel%29.pdf
13. Sri Mastuti. (2022). Potensi Bakteriosin pada Balteri Asam Laktat terhadap Staphylococcus aureus dan Escherichia coli. Jurnal Ilmiah Kesehatan Sandi Husada, 11 (1), 25-30. https://akper-sandikarsa.e-journal.id/JIKSH/article/download/650/459/#:~:text=Mekanisme%20kerja%20dari%20bakteriosin%20diperoleh,tidak%20toksin%20bagi%20yang%20mengkonsumsi
14. Y. Nurraifah, I. I. Arief, & N. Ulupi. (2021). Penggunaan Bakteriosin yang Diproduksi oleh Lactobacillus plantarum sebagai Pengawet Alami untuk Daging Ayam yang Disimpan di Suhu Ruang. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan, 9 (1), 7-14. https://journal.ipb.ac.id/index.php/ipthp/article/download/34440/21098/
15. Frans U. D., Angela N. D., Imanuel B., Annytha I. R. D., Nancy D. F. K. F., & Nemay A. N. (2019). Uji Aktivitas Antimikroba Bakteri Asam Laktat Cairan Rumen Terhadap Pertumbuhan *Salmonella Enteriditis, escheriichia coli* dan *Sthaphulococcus aureus* Menggunakan Metode Difusi Air Sumur Agar. https://media.neliti.com/media/publications/298668-uji-aktivitas-antimikroba-bakteri-asam-l-0064bfcd.pdf
16. Nadzifah & Mafrudhotin. (2022). Produksi dan karkterisasi bakteriosin lakctobacillus plntarum fncc 0026 yang diisolasi dari asinan sayur, Universitas Islam Negeri Maulana Malik Ibrahim. http://etheses.uin-malang.ac.id/42094/

Doi: 10.18196/umygrace.v4i1.666

17. Allysa S., Adi I. C., & Sarasati W. (2022). Aktivitas Antibakteri dari Bakteriosin Lactobacillus SPP. Terhadap Bakteri Resisten. Jurnal Sain Veteriner. (Vol. 40, No. 1). https://journal.ugm.ac.id/jsv/article/view/67927/0
18. Rafika S., Lia D., & Pratiwi A. (2016). Skrining Aktivitas dari Minuman *Ce Hun Tiau*. Pharmaceutical Sciences and Research (PSR). (Vol. 3, No. 2). https://scholarhub.ui.ac.id/psr/vol3/iss2/4/
19. Rofiq S. & Tawardi. (2015). Isolasi dan Karakterisasi Bakteriosin yang Dihasilkan oleh *Lactobacillus lactis* dari Sedimen Laut. JPB Kelautan dan Perikanan, 10 (1), 11-18.
20. Windy A., Rafika S., & Pratiwi A. (2018). Optimalisasi Aktivitas Bakteriosin yang Dihasilkan oleh *Lactobacillus casei* dari Sotong Kering. Jurnal Pendidikan Informatika dan Sains. (Vol. 7, No. 2). https://journal.ikippgriptk.ac.id/index.php/saintek/article/view/1041
21. Ni P. I. I. P., I Wayan S., & I Nyoman S. (2017). Karakterisasi Fisikokimia dan Uji Aktivitas Bakteriosin dari Bakteri Asam Laktat Isolator 13 B Hasil Isolasi Kolon Sapi Bali. Indonesia Medicus Veterinus, 6 (4), 278-287. http://download.garuda.kemdikbud.go.id/article.php?article=1359827&val=974&title=Karakterisasi%20Fisikokimia%20dan%20Uji%20Aktivitas%20Bakteriosin%20dari%20Bakteri%20Asam%20Laktat%20Isolat%2013%20B%20Hasil%20Isolasi%20Kolon%20Sapi%20Bali
22. Kusmiati & Amarila M. (2002). Aktivitas Bakteriosin dari Bakteri *Leuconostoc mesenteroides* Pbac1 pada Berbagai Media. MAKARA, (Vol.6, No. 1). https://lib.ui.ac.id/detail?id=117239&lokasi=lokal#
23. Fuqing H., Kunling T., Yayong L., Yanhong C., Tianwei W., Cui M., Jie Z., & Jin Z. (2021). Bacteriocins: Potential for Human Health. Oxidative Medicine and Cellular Longevity. https://onlinelibrary.wiley.com/doi/epdf/10.1155/2021/5518825
24. Irma S. & Risa N. (2017). Skrining Fotokimia dan Uji Aktivitas Antibakteri Ekstrak n-Heksan dan Metanol dari Daun Tutup Bumi (*Elephantopus scaber*) Terhadap Pertumbuhan Bakteri MRSA. PROSIDING Seminar Nasional Biotik, 5 (1), 397-402. https://jurnal.ar-raniry.ac.id/index.php/PBiotik/article/view/2179/1627
25. Risa N. & Yunita. (2012). The Potent of Methanol Extracts of Cashew (*Anacardium occidentale L.*) Against MRSA. Jurnal Natural, 12 (2), 40-43. https://www.neliti.com/publications/114908/the-potent-of-methanol-extracts-of-cashew-anacardium-occidentale-l-against-methi
26. Dwi H., Mades F., & Rhini F. (2010). Daya Hambat Sari Tanaman Obat Terhadap Pertumbuhan Bakteri Strain MRSA. Jurnal Sainstek IAIN Batusangkar, 2 (2), 125-129. https://www.neliti.com/publications/129418/daya-hambat-sari-tanaman-obat-terhadap-pertumbuhan-bakteri-strain-methicillin-re
27. Sugireng & Tiara M. (2020). Skrining bakteri penghasil senyawa metabolit anti-mrsa yang bersimbiosis dengan *Holothuria scabra* asal perairan tanjung tiram. BIOMA: jurnal biologi makassar, 5 (1), 34-39. https://media.neliti.com/media/publications/326542-skrining-bakteri-penghasil-senyawa-metab-77be13cc.pdf

Doi: 10.18196/umygrace.v4i1.667

Optimization of pH to Bacteriocin Production by *Lactobacillus Acidophillus Against Methicillin-Resistant Staphylococcus Aureus*

***Fajar* Rivaldi**[1, a)], **and *Lilis* Suryani**[2, b)]
[1] Medicine Studies, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183
[2] Department of Microbiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183

Email: fajar.rivaldi.fkik22@mail.umy.ac.id[a]; lilis.suryani@umy.ac.id[b]

ABSTRACT

Introduction: *Methicillin-Resistant Staphylococcus aureus (MRSA)* is a strain of *Staphylococcus aureus* that has developed resistance to the antibiotic's methicillin and beta lactams. MRSA has infected approximately 11 million people, and causes skin and soft tissue infections, bacteremia, pneumonia, osteomyelitis, endocarditis, meningitis, and sepsis. MRSA can be transmitted through hands, contact between patients and other people, nurses or doctors who do not wash their hands, and even health workers who have MRSA colonies on their hands and front of the nose without symptoms. **Purpose**: This study aims to determine the effect of pH on the activity of bacteriocins produced by *Lactobacillus Acidophilus* against MRSA. **Methods**: This research uses quasi-experimental laboratory methods. *Lactobacillus acidophilus* was grown in liquid MRS media for 48 hours. Bacteriocin was obtained by centrifuging liquid MRS containing bacteria at a speed of 6000 rpm for 40 minutes. The liquid formed was tested for its antibacterial effect against MRSA bacteria using the Kirby Bauer method. Bacteriocins were treated with various different pHs, that is; 2, 4, 6, 8, 10. The diameter of the inhibition zone formed during the testing process is measured in millimeters. Data were analyzed using the ANOVA method. **Finding**: The bacteriocin activity produced by Lactobacillus acidophilus against MRSA showed an average diameter of the inhibition zone of 2.90 mm. The broadest spectrum of antibacterial activity was demonstrated in bacteriocins treated with pH 2. **Conclusion:** This study shows that pH does not affect the antibacterial activity of bacteriocins produced by Lactobacillus acidophilus against MRSA. **Originality/Implication**: The results of this study prove that various pH treatments do not affect the ability of bacteriocins produced by L. Acidophilus to inhibit/kill MRSA.

Keywords: MRSA, pH, Lactobacillus acidophilus

ABSTRAK

Pendahuluan: Methicillin-Resistant Staphylococcus aureus (MRSA) adalah jenis Staphylococcus aureus yang telah mengembangkan resistensi terhadap antibiotik metisilin dan beta laktam. MRSA telah menginfeksi sekitar 11 juta orang, dan menyebabkan infeksi kulit dan jaringan lunak, bakteremia, pneumonia, osteomielitis, endokarditis, meningitis, dan sepsis. MRSA dapat ditularkan melalui tangan, kontak antara pasien dengan orang lain, perawat atau dokter yang tidak mencuci tangan, dan bahkan petugas kesehatan yang memiliki koloni MRSA di tangan dan bagian depan hidung tanpa gejala. **Tujuan:** Penelitian ini bertujuan untuk mengetahui pengaruh pH terhadap aktivitas bakteriosin yang dihasilkan oleh Lactobacillus Acidophilus terhadap MRSA. **Metode:** Penelitian ini menggunakan metode laboratorium eksperimental semu. Lactobacillus acidophilus ditumbuhkan dalam media MRS cair selama 48 jam. Bakteriosin diperoleh dengan cara menyentrifugasi MRS cair yang mengandung bakteri dengan kecepatan 6000 rpm selama 40 menit. Cairan yang terbentuk diuji efek antibakterinya terhadap bakteri MRSA dengan metode Kirby Bauer. Bakteriosin diperlakukan dengan berbagai pH yang berbeda, yaitu; 2, 4, 6, 8, 10. Diameter zona hambat yang terbentuk selama proses pengujian diukur dalam satuan milimeter. Data dianalisis dengan menggunakan metode ANOVA. **Temuan:** Aktivitas bakteriosin yang dihasilkan oleh Lactobacillus acidophilus terhadap MRSA menunjukkan diameter rata-rata zona hambat sebesar 2,90 mm. Spektrum aktivitas antibakteri yang paling luas ditunjukkan pada bakteriosin yang diberi perlakuan pH 2. **Kesimpulan:** Penelitian ini menunjukkan bahwa pH tidak mempengaruhi aktivitas antibakteri bakteriosin yang diproduksi oleh Lactobacillus acidophilus terhadap MRSA. **Keaslian/Implikasi:** Hasil penelitian ini membuktikan bahwa perlakuan pH yang berbeda tidak mempengaruhi kemampuan bakteriosin yang dihasilkan L. acidophilus dalam menghambat/membunuh MRSA.

Keywords: MRSA, pH, Lactobacillus acidophilus

Doi: 10.18196/umygrace.v4i1.667

INTRODUCTION

Staphylococcus aureus bacteria, which are one of the normal florae on the skin and anterior nasal passages, can become pathogenic when the patient's immune system is decreased, and about 30% of healthy adults have these bacteria inside their nose and 20% on the surface of their skin (Suyasa & Mastra, 2020). Due to the ability of S. aureus bacteria to produce an enzyme encoded by the mecA gene, these bacteria are becoming more common worldwide and are known as MRSA, or antibiotic-resistant *Staphylococcus aureus* (Prasetya et al., 2019). MRSA *(Methicillin-Resistant Staphylococcus aureu*s) is a strain of S. aureus that is resistant to methicillin and *β-lactam* antibiotics (Rahman et al., 2023). The 76kDa gene in MRSA bacteria expresses the *penicillin-binding protein* (PBP2a) enzyme that allows it to hydrolyze most *β-lactam* antibiotics. Nearly 11 million people are infected by this bacterium with skin and soft tissue infections, bacteremia, pneumonia, osteomyelitis, endocarditis, meningitis, and sepsis (Bhakyasharee & Khanabirran, 2018).

Lactobacillus acidophilus is one of the producers of lactic acid bacteria (LAB), which consists of rod-shaped or cocci (round) gram-positive bacteria that do not have spores and produces its main product, lactic acid, from carbohydrate fermentation. *L. acidophilus* can be used in the fermentation industry to produce organic acids and bacteriocins that help prevent the growth of microorganisms (Ningrumsari & Herlinawati, 2019). Most *L.acidophilus* strains are microaerobic bacteria, which grow better in anaerobic environments, or at 5–10% CO2, than in aerobic environments. *L.acidophilus* lacks heat resistance, and its ideal pH is 5.5–6.0 (Gao et al., 2022).

Antimicrobial peptides called bacteriocins can kill or stop the growth of other bacteria. In addition, bacteriocins have amino acid sequences and biochemical properties that are not yet fully known. Antimicrobial compounds that are not yet fully known are referred to as *Bacteriocin-Like Inhibitory Substances* (BLIS). It is known that bacteriocins have bactericidal properties against species that are phylogenetically close to the producing species (Purwaningsih, 2018).

Bacteriocins are also known as antimicrobial peptides that are synthesized in ribosomes, have a high degree of target specificity towards bacterial strains that are close to them, and have antimicrobial activity at concentrations as low as picomolar to nanomolar (Woraprayote, 2016). Bacteriocins meet several criteria, including being a protein, being bactericidal, and having a specific binding site to the target bacteria. The gene encoding bacteriocin is in the plasmid and functions against bacteria that are close in phylogeny but does not kill the bacteria that produce it (Purwaningsih, 2018). Several variables, including pH, temperature, enzymes, and organic solvents, generally affect bacteriocin activity (Senbagam et al., 2013). In Al-Mu'minun verses 12–14, Allah SWT explains how all living things develop, from living cells (germs or sperm) that are unicellular (single-celled) microscopic to new creatures (humans) that are multicellular (many-celled) macro (Subandi & Latifah, 2014).

LITERATURE REVIEW

(1) Methicillin-Resistant Staphylococcus aureus (MRSA)

Infection of Staphylococcus aureus by the bacteria Methicillin-resistant Staphylococcus aureus, or MRSA, is a bacteria that is resistant to some antibiotics, making treatment extremely challenging. MRSA bacterial infections are now a major worldwide issue that is present in many nations. Globally, there are still a lot of cases, and they are continually growing (Salsabil et al., 2022).

According to data from the World Health Organization, MRSA is the second infectious germ that requires further study and development in order to produce new medications. Numerous illnesses, including skin infections, wounds, lung infections, pneumonia, and bloodstream infections that can result in sepsis or even death, can be brought on by MRSA infections (Almira et al., 2022). Globally, MRSA is thought to be the primary source of nosocomial infections. It was that people who had MRSA bacterial infections in their bodies had a 64% higher mortality rate than people who had germs that did not withstand a range of medications. As a result, society depends heavily on the MRSA bacterial infection being treated (Salsabil et al., 2022).

(2) L. achidophilus

Bacteria of the type L. acidophilus were used in this study. In the MRSA medium, isolate regeneration takes place. Regeneration is the updating and expansion of bacterial cell proliferation. The reason this is crucial is that it will create fresh, vibrant bacterial breeds that are prepared for the best possible fermentation process (Freire et al., 2015).

The bacteria Lactobacillus acidophilus can be used to make probiotics. Because of the bacterial ability to convert sugar into lactic acid, lactic acid bacteria (BAL) are widely used in the fermentation process to produce lactic Acid. With their ability to transform sources of sugar, especially lactose, into lactate acid, Lactobacillus bacteria can be distinguished from other acid-producing bacteria. Strain Lactobacillus has high commercial value due to its high tolerance to acid, yield, and productivity. In addition, the strain of Lactobacillus can be modified to make selective L/D acid lactate (Pujasari, 2019).

(3) Bacteriocins

Many bacteria and some archaea create harmful proteins called bacteriocins that are meant to stop the growth of strains that are similar to or closely related to one another. At specific doses, ribosomes with antimicrobial properties produce these proteins (Negash & Tsehai, 2020).

There are numerous advantages to bacteriocins that can be utilized. They can withstand high thermal pressures and remain active. Furthermore, there is no taste, color, or smell to these antimicrobial proteins. Because of their protein composition, bacteriosin fragments are quickly broken down by proteolytic enzymes and are not found for very long in the human body or in the natural world. As a result, there is less chance that fragmented bacteriosins and target strains will interact. Because of this benefit, bacteriosine has gained popularity as an additive in the culinary, medicinal, and agricultural sectors (Negash & Tsehai, 2020).

METHODS

A. Study design

This research uses quantitative methods and is a form of laboratory experiment. It was conducted in May–June 2024 at the Microbiology Laboratory of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta (FKIK UMY). This research has obtained permission from the Health Research Ethics Committee of FKIK UMY, No. 046/EC-EXEM-KEPK FKIK UMY/V/2024.

B. Samples

The sample for this research is Lactobacillus acidophilus, obtained from the PAU UGM Building, Jl. Teknika Utara, Yogyakarta Special Region 55281. *L. acidophilus* obtained is a type that is ready to be treated, and then in the microbiology laboratory, planting is carried out on liquid media and put into an anaerobic container. After that, incubation was carried out for 48 hours at 37 °C.

C. Tools and Materials

The tools used in this study include: Petri dish (Iwaki pyrex), Ose needle, object glass, test tube, erlemeyer tube (Iwaki pyrex), beaker glass (Iwaki pyrex), autoclave, microscope, incubator, Biological Safety Cabinet, bunsen, refrigerator, centrifugation, water bath and caliper. The materials used in this study include: Lactobacillus acidophilus isolate, De Man Rogosa and Sharpe (MRS) agar media, De Man Rogosa and Sharpe (MRS) broth media, distilled water, crystal violet, iodine, 95% ethanol, safranin, phosphate spar, NaOH, HCl, Mueller Hinton Agar (MHA), Trypticase Soy Agar (TSA).

D. *Lactobacillus acidophilus* antibacterial preparation

Each *L. acidophilus* bacterial isolate isolated from liquid MRS was inoculated into 10 ml of MRSB. Then it was incubated for 48 hours at 37ºC in an anaerobic atmosphere. Then it was centrifuged at 6000 rpm for 30 minutes and 2 repetitions. The first treatment was as much as 3 mL of bacteriocin, and the second treatment was 2 mL of bacteriocin, which was then put in an effluent tube. The resulting supernatant is a crude extract of antibacterial *L. acidophilus*. The supernatant is ready to be tested for antibacterial power against *Methicillin-Resistant Staphylococcus aureus* (MRSA) test bacteria.

E. Antibacterial Test of *Lactobacillus acidophilus* against MRSA

An antimicrobial activity test was conducted on MHA media. Colonies of test bacteria (*Methicillin-Resistant Staphylococcus aureus)* were incubated for 24 hours, and then some were taken with a sterile ose and suspended into a test tube containing 5 mL of a sterile physiological NaCl solution. The turbidity of the suspension was compared with the 0.5% McFarland turbidity standard, which is equivalent to 1.5 × 108 CFU/mL on a McFarland densitometer. Then the treatment involved preparing a sterile cotton swab dipped in a suspension of test bacteria, which was then scraped evenly on the entire surface of the MHA medium. Then let it stand for 5–15 minutes so that the suspension can seep into the MHA media. Paper discs were dripped with 20 µl of *L. acidophilus* bacterial supernatant suspension. The MHA media was incubated at 37 °C for 24 hours in an aerobic atmosphere. The diameter of the inhibition zone formed was measured in millimeters to determine the antimicrobial activity of *L. acidophilus* against the test bacteria.

F. Research Procedure

1) *Lactobacillus acidophilus* Bacterial Culture Rejuvenation.

   Lactobacillus acidophilus culture was renewed through subculture on MRS agar media using the Streak Plate method. Bacterial colonies were inoculated into MRS agar media using a sterile loop by streaking. The MRS agar medium was then incubated at 37°C for 48 hours under anaerobic conditions. The colonies that grow are then identified using gram staining.

2) Bacteria were identified using the Gram staining method.

   The procedure begins by adding one drop of crystal violet to the preparation that has been smeared with a 24-hour-old *Lactobacillus acidophilus* isolate. The preparation was left for 1 minute, then washed with distilled water. Next, one drop of iodine is added, left for 2 minutes, then rinsed with distilled water. The preparations were washed again with 95% ethanol and rinsed with running water. The isolate was then stained with safranin and rinsed with running water. The preparations were dried at room temperature and observed under a microscope with 1000x magnification.

3) Isolation of Bacteriocins Produced by *Lactobacillus acidophilus.*

Lactobacillus acidophilus bacterial isolates that had grown on MRS agar for 48 hours were inoculated into 10 ml of MRS broth media and incubated for 48 hours at 37°C under anaerobic conditions. After that, the culture was centrifuged at 6000 rpm for 30 minutes twice. 5 ml of supernatant fluid was taken and put into an Eppendorf tube. This liquid is used as a source of bacteriocin and is ready to be tested for its antibacterial activity.

4) pH treatment of bacteriocins.

A total of 5 ml of bacteriocin was divided into 5 groups, each group was given a different pH treatment, namely 2, 4, 6, 8, and 10. Each pH treatment was repeated twice. pH treatment was carried out by adding bacteriocin to an Eppendorf tube with a volume of 1.25 ml for each treatment. Then, the bacteriocin is dropped with 1N HCl or NaOH according to the desired pH. After that, the antibacterial activity of bacteriocins against *Methicillin-resistant Staphylococcus aureus* (MRSA) was tested using the Kirby Bauer method.

5) *Methicillin-resistant Staphylococcus aureus* (MRSA) Test Bacteria Preparation.

MRSA test bacteria were inoculated into TSA media and incubated at 37°C for 24 hours under aerobic conditions. To prepare the MRSA inoculum, several MRSA colonies were taken from the TSA medium using a loop and transferred into 2 ml of BHI medium. Inoculum turbidity was adjusted to the 0.5% McFarland standard which corresponds to 1.5 × 10^8 CFU/mL.

6) Antibacterial Testing of Bacteriocins against *Methicillin-resistant Staphylococcus aureus* (MRSA) using the Kirby Bauer Method.

MRSA test bacteria with a concentration of 1.5 × 10^8 CFU/mL were grown on Mueller Hinton Agar media using sterile cotton swabs. A sterile cotton swab is dipped in the test bacterial suspension and then rubbed evenly over the entire surface of the MHA media, then left for 5-15 minutes so that the suspension can absorb into the media. The paper disc was dripped with 20 µl of bacteriocin supernatant suspension that had been treated with various heating temperatures. The discs were then placed on MHA media which had been inoculated with the test bacteria and incubated at 37°C for 24 hours under aerobic conditions. The diameter of the inhibition zone formed is measured with a caliper in millimeters. The size of this inhibition zone is used to assess the antimicrobial activity of bacteriocins from *Lactobacillus acidophilus* against MRSA bacteria.

7) Data Analysis.

Data on the diameter of the inhibition zone from each treatment were analyzed using the ANOVA test to evaluate the effect of pH treatment on the activity of bacteriocins produced by *Lactobacillus acidophilus* against MRSA.

RESULT

Based on Figure 1, it shows the measurement of the inhibition zone of bacteriocin activity obtained by *Lactobacillus acidophilus* against *Methicillin-Resistant Staphylococcus aureus* (MRSA) with an average inhibition zone of 2.90 millimetres. This study proves that bacteriocins produced by lactic acid bacteria isolated from liquid MRS have activity against gram-positive and gram-negative bacteria.

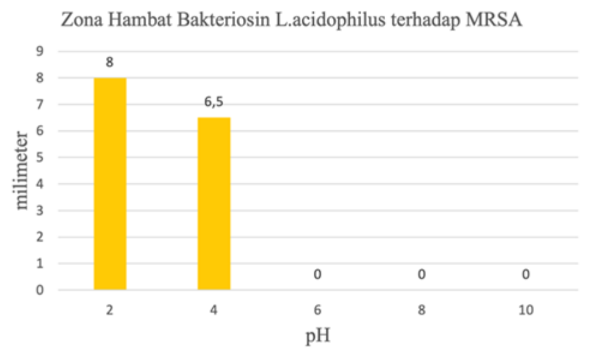


Figure 1. Diagram of the Zone of Inhibition of *L. acidophilus* Bacteriocin Against MRSA

The results from the observation of Figure 1 show that the average zone of bacteriocin inhibition in the treatment of pH 4; 6; 8; and 10 is 2.90 millimeters, and the zone of bacteriocin inhibition, respectively, at pH 2 is 8 millimeters, pH 4 is 6.50 millimeters, pH 6 is 0 millimeters, pH 8 is 0 millimeters, and pH 10 is 0 millimeters.

From this study, the widest inhibition zone spectrum was obtained at pH 2 with an inhibition zone of 8 millimeters, and the narrowest inhibition zone spectrum was at pH 6, 8, and 10 with an inhibition zone of 0 millimeters. The data obtained were then tested using a one-way ANOVA test with two repetitions of treatment, and data measurement was only done after treatment.

The statistical test results to see whether there is an effect of pH on the bacteriocin activity of *Lactobacillus acidophilus* against *Methicillin-Resistant Staphylococcus aureus* (MRSA) showed the following results:

1. The results of the Kruskal-Wallis test can be seen in Figure 2

pH	N	Mean	Std. Deviation	P
pH 2	2	8.000	1.414	
pH 4	2	6.500	0.707	
pH 6	2	0.000	0.000	0.069
pH 8	2	0.000	0.000	
pH 10	2	0.000	0.000	

Figure 2. Kruskal-Walli's test results

Based on Figure 2, the results showed that overall, there was no difference in the activity of *Lactobacillus acidophilus* bacteria against MRSA, with a P value of 0.069. Thus, pH has no significant effect on the activity of *Lactobacillus acidophilus* bacteria against MRSA.

2. The results of the Mann-Whitney test can be seen in Figure 3

pH		P	Keterangan
2	4	0.221	Tidak terdapat perbedaan
	6	0.102	Tidak terdapat perbedaan
	8	0.102	Tidak terdapat perbedaan
	10	0.102	Tidak terdapat perbedaan
4	6	0.102	Tidak terdapat perbedaan
	8	0.102	Tidak terdapat perbedaan
	10	0.102	Tidak terdapat perbedaan
6	8	1.000	Tidak terdapat perbedaan
	10	1.000	Tidak terdapat perbedaan
8	10	1.000	Tidak terdapat perbedaan

Figure 3. Mann-Whitney test results

Based on Figure 3, the results showed that pH did not affect the bacteriocin activity of *Lactobacillus acidophilus* against MRSA.

DISCUSSION

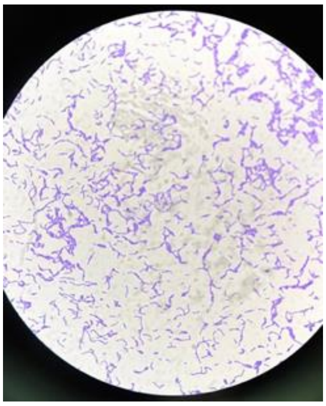


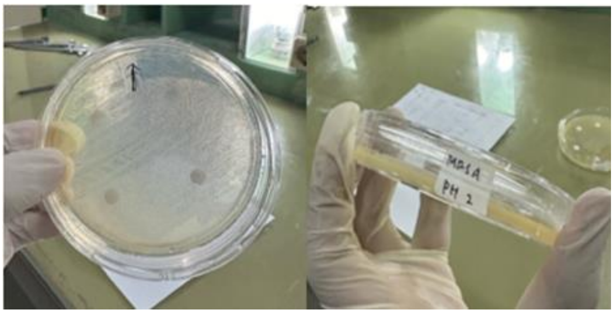
Figure 4. Microscopic staining of L. acidophilus isolates by Gram technique

This study proves that bacteriocin from *L. acidophilus* has antibacterial power against *Methicillin-Resistant Staphylococcus Aureus* (MRSA) bacteria, and this study proves that pH does not affect the activity of bacteriocin produced by *L. acidophilus* against MRSA. Diameter of

bacteriocin inhibition zone from *L. acidophilus* isolate bacteria with an average of 2.90 mm against *Methicillin-Resistant Staphylococcus aureus* (MRSA) test bacteria.

Based on Figure 4, the results of staining the isolation of L. acidophilus bacteria with the gram technique show that *L. acidophilus* bacteria have microscopic characteristics of rod-shaped cells and are gram-positive.

Figure 5. Diameter of Zone of Inhibition of



L.acidophilus bacteriocin against MRSA at pH2

Based on Figure 5, it shows that the diameter of the inhibition zone of *L. acidophilus* bacteriocin against MRSA at pH 2 with an inhibition zone formed of 8 mm is the optimal inhibition zone formation.

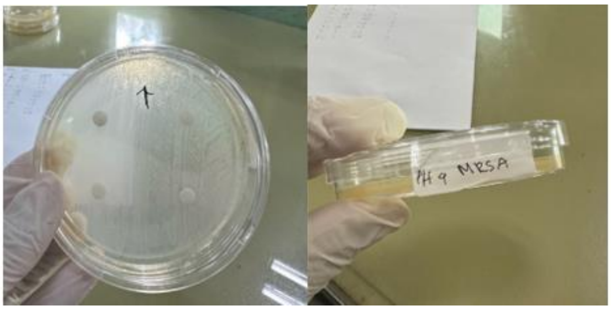


Figure 6. Diameter of Zone of Inhibition of L.acidophilus bacteriocin against MRSA at pH

Based on Figure 6, it shows that the diameter of the inhibition zone of *L. acidophilus* bacteriocin against MRSA at pH 4 is 6.50 mm.

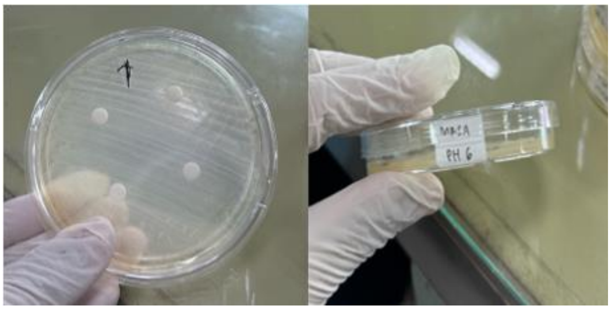


Figure 7. Diameter of Zone of Inhibition of *L. acidophilus* Bacteriocin Against MRSA at pH 6

Based on Figure 7, it shows that the diameter of the inhibition zone of *L. acidophilus* bacteriocin against MRSA at pH 6 with an inhibition zone formed of 0 mm is the narrowest inhibition zone.

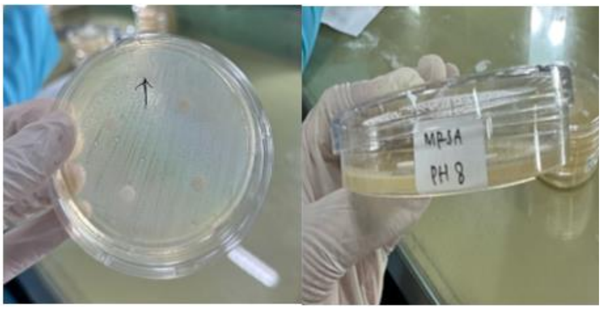


Figure 8. Diameter of Zone of Inhibition of *L. acidophilus* bacteriocin against MRSA at pH 8

Based on Figure 8, it shows that the diameter of the inhibition zone of *L. acidophilus* bacteriocin against MRSA at pH 8 with an inhibition zone formed of 0 mm is the narrowest inhibition zone.

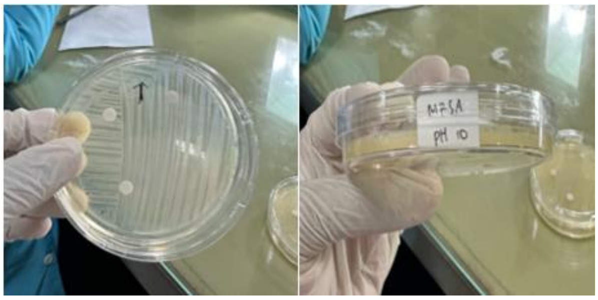


Figure 9. Diameter of Zone of Inhibition of *L. acidophilus* bacteriocin against MRSA at pH 10

Based on Figure 9, it shows that the diameter of the inhibition zone of *L. acidophilus* bacteriocin against MRSA at pH 10 with an inhibition zone formed of 0 mm is the narrowest inhibition zone.

There are four categories of antimicrobial inhibition zone activity: weak (less than 5 mm), moderate (between 5 and 10 mm), strong (between 10 and 20 mm), and very strong (between 20 and 30 mm). The clear zone created around the disc paper indicates blocking antimicrobial activity. The area that inhibits bacterial growth has a length measured in millimeters (Datta et al., 2019). This study proves that the existence of temperature variations does not have a significant effect on the diameter of the inhibition zone produced by bacteriocins against the growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA). The average value of the diameter of the inhibition zone formed by bacteriocin in all temperature treatments is 2.90 mm; the size of this diameter is included in the group of weak antibacterial activity with a diameter range of less than 5 mm.

Inhibition of protein synthesis by bioactive compounds inhibits bacterial growth or bacterial death. Gram-positive and gram-negative bacteria are not equally resistant to antibacterials. Cell wall structure, such as the amount of peptidoglycan (including the presence of receptors, pores, and lipids), cross-linking properties, and autolic enzyme activity, are responsible for the differences in sensitivity of gram-negative and gram-positive bacteria. These components determine penetration, binding, and antimicrobial activity (Wirjatmadja et al., 2022). The mechanisms of antibacterial inhibition of bacterial growth include cell wall damage, which causes lysis or inhibition of cell wall synthesis; permeability of the cytoplasmic membrane, which allows food materials through the cell wall; denaturation of cell proteins; and destruction of metabolic systems within the cell by stopping the action of intracellular enzymes (Hamzah, 2019).

Bacteriocins, hydrogen peroxide, and lactic acid are produced by *L. acidophilus*. It opposes gram-negative bacteria, *Staphylococcus aureus*, and *Salmonella sp.*, food-stripping bacteria. *Acidophilucin, acidocin, acidophilicin,* and a*cidophilin* are the names of *L. acidophilus* bacteriocins as antimicrobials that can be used in biopreservatives and fermented milk products that can stop pathogenic bacteria (Paryati et al., 2022). *L. acidophilus* bacteria can block fungi, gram-positive bacteria, and gram-negative bacteria due to its broad spectrum (Zhao et al., 2015).

Bacteriocins impair the permeability of the target cytoplasmic membrane by forming a pore in the cell membrane. This can lead to metabolite leakage and eliminate the proton motive force, or proton motive force (PMF). PMF is the cytoplasmic membrane electrochemical gradient that regulates *adenosine triphosphate* (ATP) synthesis. A decrease in PMF will lead to cell death due to the cessation of energy generation. The cytoplasmic membrane of bacterial cells serves as the main target of bacteriocins. The main receptor of bacteriocin and part of the pore formation process are the negatively charged cytoplasmic membrane lipids. Bacteria will bind to the destination cell membrane through electrostatic interactions (Basarang, 2013).

CONCLUSION AND RECOMENDATION

This study shows that different pH experiments do not affect the antibacterial activity of bacteriocins produced by L. acidophilus against MRSA. Bacteriocins produced by L. acidophilus have antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA) bacteria. The average inhibition zone of *L. acidophilus* bacteriocin in pH 2; 4; 6; 8; and 10 treatments was 2.90 mm.

The treatment of various pH did not affect the bacterial activity of bacteriocins produced by *L.acidophilus* against MRSA. The optimal temperature to obtain antibacterial activity of bacteriocins produced by *L.acidophilus* was pH 2, with an inhibition zone diameter of 8 milimeters.

The presence of antibacterial activity of bacteriocins can be developed of further research, to obtaincomplete information about the utilization of bacteriocins as antibacterial against pathogenic bacteri, especially those that are resistant to various antibiotic substances.

Acknowledgments. during the research process, of course, it cannot be separated from guidance, assistance, and motivation from related parties. Therefore, the author would like to thank Dr. Dra. Lilis Suryani, M. Kes, and Lia Fitriana, S.P., who have helped during the research.

REFERENCE

1. Basarang, M. (2013). Pengaruh Bakteriosin dari Streptococcus thermophilus sebagai Pengawet terhadap Lama Penyimpanan Dangke (Doctoral dissertation, Universitas Hasanuddin)
2. Bhakyashree, K., & Kannabiran, K. (2020). Actinomycetes mediated targeting of drug-resistant MRSA pathogens. Journal of King Saud University-Science, 32(1), 260-264.
3. Datta, F. U., Daki, A. N., Benu, I., Detha, A. I. R., Foeh, N. D., & Ndaong, N. A. (2019). Uji aktivitas antimikroba bakteri asam laktat cairan rumen terhadap pertumbuhan Salmonella enteritidis, Bacillus cereus, Escherichia coli dan Staphylococcus aureus menggunakan metode difusi sumur agar. Jurnal Kajian Veteriner, 66-85.
4. Freire, A. L., Ramos, C. L., & Schwan, R. F. (2015). Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. *Food Research International*, *76*, 787-795.
5. Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L., & Li, P. (2022). The functional roles of Lactobacillus acidophilus in different physiological and pathological processes. Journal of microbiology and biotechnology, 32(10), 1226.
6. Hamzah, A. (2019). Analisis in vitro aktivitas antibakteri daun sisik naga (Drymoglossum pilosellaoides) terhadap bakteri Vibrio harveyi dan Vibrio parahaemolyticus. Journal of Aquaculture and Fish Health, 8(2), 86-91.
7. Negash, A. W., & Tsehai, B. A. (2020). Current applications of bacteriocin. *International Journal of Microbiology*, *2020*(1), 4374891.
8. Ningrumsari, I., & Herlinawati, L. (2019). Peranan Lactobacillus acidophilus dalam pakan terfermentasi untuk meningkatkan kualitas daging ayam broiler (protein, kolesterol). Jurnal Pertanian, 10(2), 93-101.
9. Paryati, S. P. Y., Raja, E. N. L., & Andana, P. (2022). Perbandingan Efektivitas Bakteriosin Lactobacillus acidophillus ATCC 4356 dengan Nisin pada Pertumbuhan Salmonella typhi ATCC 6539. Medika Kartika: Jurnal Kedokteran dan Kesehatan, 5(2), 162-174.
10. Prasetya, Y. A., Nisyak, K., & Iftitah, E. D. (2019, October). Aktivitas Nanokomposit ZNO-Ag dalam Menghambat Pertumbuhan Bakteri Methicillin Resistant Staphylococcus aureus (MRSA). In Seminar Nasional Sains & Entrepreneurship (Vol. 1, No. 1).
11. Pujasari, A. (2019). *Pengaruh jenis bakteri asam laktat dan konsentrasi inokulum terhadap produksi asam laktat dari air kelapa* (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).
12. Purwaningsih, N. (2018). Karakterisasi Bakteriosin Yang Dihasilkan Oleh Lactobacillus Pentosus K 50 Sebagai Agen Biopreservatif Pangan (Doctoral dissertation, Universitas Brawijaya).
13. Rahman, I. W., Arfani, N., Rafika, R., & Tadoda, J. V. (2023). Deteksi bakteri MRSA methicillin-resistant Staphylococcus aureus pada sampel darah pasien rawat inap. Jurnal Ilmu Alam dan Lingkungan, 14(1).
14. Salsabil, A. D., Rochmanti, M., & Widodo, A. D. W. (2022). Vancomycin Monotherapy vs Alternative Antibiotics for MRSA Patients: A Systematic Review. *Vancomycin Monotherapy vs Alternative Antibiotics for MRSA Patients: A Systematic Review*, *92*(1), 12-12.
15. Senbagam, D., Gurusamy, R., & Senthilkumar, B. (2013). Physical chemical and biological characterization of a new bacteriocin produced by Bacillus cereus NS02. Asian Pacific journal of tropical medicine, 6(12), 934-941.
16. Subandi, M., & Latifah, P. (2014). Mikrobiologi: Kajian dalam Perspektif Islam Edisi Revisi.
17. Suyasa, I. B. O. (2020). Gambaran Methicillin Resistant Staphylococcus aureus (MRSA) Pada Petugas Kesehatan RSUD Wangaya Kota Denpasar. Meditory: The Journal of Medical Laboratory, 8(1), 46-52.
18. Wirjatmadja, R., Kurniasari, P. N. I., Wibisono, F. J., & Kurnianto, A. (2022). Efektivitas antibakteri ekstrak daun sisik naga (Drymoglossum piloselloides) terhadap bakteri MRSA (methicilin resistant Staphylococcus aureus) dan Eschericia coli. VITEK: Bidang Kedokteran Hewan, 12(2), 26-35.
19. Woraprayote, W., Malila, Y., Sorapukdee, S., Swetwiwathana, A., Benjakul, S., & Visessanguan, W. (2016). Bacteriocins from lactic acid bacteria and their applications in meat and meat products. Meat Science, 120, 118-132.
20. Zhao, R., Duan, G., Yang, T., Niu, S., & Wang, Y. (2015). Purification, characterization and antibacterial mechanism of bacteriocin from Lactobacillus acidophilus XH1. Tropical Journal of Pharmaceutical Research, 14(6), 989-995.

 Doi: 10.18196/umygrace.v4i1.668

Level of Knowledge and Attitude of Nurses as First esponders in an In-Hospital Cardiac Arrest Event

Sandi Ihsan Rafiqi[a], and Azizah Khoiriyati[b]
School of Nursing, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Bantul, Indonesia, 55183
Email: Sandiihsan7@gmail.com[a], azizah.khoiriyati@umy.ac.id[b]

ABSTRACT

Introduction - In-hospital cardiac arrest (IHCA) is an emergency condition that can threaten a person's life if not treated immediately. Sufficient nurses' knowledge and attitude may lead to successful IHCA prevention and handling. Purpose - This study aims to determine the level of knowledge and attitude of nurses at PKU Muhammadiyah Hospital Yogyakarta in handling IHCA. Methodology - Research method uses a quantitative descriptive research design. One hundred nurses from the adult care ward of a hospital in Yogyakarta, Indonesia were recruited using the purposive sampling technique. This research was conducted from December 2023 to March 2024. The research instrument used was a nurse level of knowledge questionnaire and a nurse attitude questionnaire. Findings - Most of the nurses has good knowledge (73 respondents (73%)) and attitude (94 respondents (94%)) as first responders during cardiac arrest events. There are still errors in answering the BLS algorithm points and airway and breathing management. Conclusion - Level of knowledge and attitude of nurses is in the good category. This research can be used as material for evaluating nurses when treating cardiac arrest patients in hospitals.
Keywords: Attitude, Cardiac Arrest, First Responder, In-Hospital Cardiac Arrest, Knowledge Level.

ABSTRAK

Pendahuluan - In-hospital cardiac arrest (IHCA) adalah kondisi darurat yang dapat mengancam nyawa seseorang jika tidak dirawat segera. Pengetahuan dan sikap perawat yang memadai dapat menyebabkan pencegahan dan pengolahan IHCA yang sukses. Tujuan - penelitian ini bertujuan untuk menentukan tingkat pengetahuan dan sikap perawat di PKU Muhammadiyah Hospital Yogyakarta dalam menangani IHCA. Metode - Metode penelitian menggunakan desain penelitian deskriptif kuantitatif. Seratus perawat dari departemen perawatan dewasa di rumah sakit di Yogyakarta, Indonesia. Pengamilan sample menggunakan teknik Purposive Sampling. Penelitian ini dilakukan dari Desember 2023 hingga Maret 2024. Instrumen penelitian yang digunakan adalah kuesioner tingkat pengetahuan perawat dan kuesional sikap perawat. Hasil - Tingkat pengetahuan dan sikap perawat berada dalam kategori yang baik. Penelitian ini dapat digunakan sebagai bahan untuk mengevaluasi perawat saat mengobati pasien berhenti jantung di rumah sakit. Kesimpulan - Tingkat pengetahuan dan sikap perawat berada dalam kategori yang baik. Penelitian ini dapat digunakan sebagai bahan untuk mengevaluasi perawat saat mengobati pasien berhenti jantung di rumah sakit. *Keywords: Henti Jantung, Penolong pertama, In-Hospital Cardiac Arest, Tingkat pengetahuan, sikap.*

INTRODUCTION

Cardiac arrest is a condition where cardiac output is insufficient to supply oxygen to the brain and important vital organs in the body. This cardiac arrest condition can be recovered with appropriate action, but failure to take appropriate action can cause death or permanent damage (Ismiroja et al., 2018). The World Health Organization (WHO) says that heart disease accounts for 18.6 million of the 29.5 million deaths; this is the number one largest contributor to death in the world. (WHO, 2016). The incidence of cardiac arrest in Indonesia reaches 300–350 thousand cases every year, if it can be estimated at around 10 per 10,000 adults under the age of 35 years (PERKI, 2017). Riskesdas data in 2018 stated that the prevalence of cardiac arrest based on expert diagnosis in Indonesia reached 1.5%, with the largest prevalence in North Kalimantan Province, which contributed 2.2%, Gorontalo 2%, and the Special Region of Yogyakarta reaching 2%.

In-Hospital Cardiac Arrest (IHCA), or cardiac arrest in the hospital, is an emergency condition in a hospital that can threaten a person's life if treatment is not carried out as soon as possible (Subhan et al., 2019). The prevalence rate of IHCA in America is estimated to reach 200 thousand people each year, with a survival rate after the event of < 20% (Darwati et al., 2019).

Before the code blue team arrives, nurses, as first responders or people who discover a cardiac arrest incident in a hospital treatment room, need to master the knowledge and ability to carry out basic life support (Dhani Irawan et al., 2021). Nurses are one of the main helpers in dealing with cases of cardiac arrest and respiratory arrest. Nurses are required to carry out treatment as soon as possible after identifying the patient (Dame et al., 2018).

Treatment for cardiac arrest should not last more than 5 minutes, so treatment for cardiac arrest needs to be done immediately (American Heart Association, 2020). The chain

Doi: 10.18196/umygrace.v4i1.668

of survival management from IHCA can prevent the worst events in cardiac arrest patients. The steps consist of early identification and response, code blue activation, high-quality heart massage, cardiac shock (defibrillation), and care after cardiac arrest (American Health Association, 2020).

Hospitals need a good system and organization to carry out cardiac arrest cases in hospitals. Apart from knowledge, the attitude of the nurses needs to be studied so that when an emergency occurs in the ward, not only the code blue team plays a role, but the nurses also need to be able to do the things that need to be done in handling patients in cases. There is still little literature that discusses the level of knowledge and attitudes of nurses in in-hospital cardiac arrest settings, so researchers want to know the level of knowledge and attitudes of nurses as first responders in in-hospital cardiac arrest events".

LITERATURE REVIEW

PKU Yogyakarta Hospital has an integrated system for handling cardiac arrests using the code blue system. In its implementation, when an arrest incident was discovered in the ward, one of the nurses activated the system with telephone code 888, and the blue team must arrive no later than 5 minutes. The success rate in performing cardiopulmonary resuscitation (CPR) is unknown, and the hospital has not carried out an evaluation regarding the level of knowledge and attitude of each nurse because hospital nurses need to have BTCLS or ATCLS certification.

In research conducted by Dhani Irawan et al. (2021), which was carried out on the Code Blue team, it showed that 64,1% f nurses had good knowledge in carrying out BLS and 87.2% had good attitudes in their attitude. Another study, which was researched by Ika Safitri et al. (2022), The results showed that the majority of respondents were in the elderly category (≥60 years) as much as 55.2%, male as much as 69%, and 72.4% showed an early ECG rhythm of asystole. The most frequent cause of cardiac arrest is non-cardiac disease (79.3%), and the majority occurs in the ICU-ICCU (55.2%). All respondents received CPR and epinephrine (100%). Defibrillation was given to 24.1%, amiodarone was given to 10.3%, and endotracheal intubation was given to 37.9% of respondents. 24.1% of respondents achieved ROSC, but five of them experienced repeated cardiac arrests and were declared dead.

METHOD

This research design uses a quantitative descriptive research design with a purposive sampling technique. This research was conducted at the PKU Muhammadiyah Yogyakarta Hospital from December 2023 to March 2024. The respondents for this research were 100 adult ward nurses. This study used a nurse knowledge level questionnaire and a nurse attitude questionnaire from (Dhani Irawan et al., 2021). Data analysis using computer program.

RESULT

After collecting data from 100 respondents, the following result were obtained:

Table 1. Frequency distribution and percentage of respondent characteristics based on gender, education, length of work, age and BLS training (n = 100)

Characteristics	Frequency	Percentage(%)
Gender		
Male	25	25%
Female	75	75%
Last Education		
D3	62	62%
D4	3	3%
S1	32	32%
S2	3	3%
Lenght of Work		
<10 year	34	34%
11-20 year	13	13%
>21 year	53	53%
Age		
<30 year	24	24%
31-40 year	22	22%
>41 year	54	54%
BLS Training		
BTCLS	86	86%
ATCLS	3	3%
Haven't attended training	11	11%

Based on Table 1, it can be seen that the gender characteristics according to the demographic data of the research respondents are mostly women, namely 75 respondents (75%). Based on the characteristics of the most recent education, the majority of respondents had a D3 nursing education, with 62 respondents (62%). Regarding work experience, the majority of respondents have worked >21 years, namely 53 respondents (53%). Characteristics of respondents based on age: most of them were >31 years old, namely 54 years (54%). Characteristics of the BLS training that has been undertaken. The majority have attended BTCLS training, with 86 respondents (86%).

Table 2. Level of nurse knowledge (n=100)

Level of Knowledge	Frequency	Percentage (%)
Good	73	73%
Enough	26	26%
Not enough	1	1%
Total	100	100%

Based on table 2, the results show that the knowledge of nurses as first responders when a cardiac arrest occurs is in the good category, namely 73 respondents (73%) of the total sample. A total of 26 respondents (26%) had sufficient knowledge, and there was 1 respondent (1%) who fell into the insufficient criteria.

Table 3. Level of nurse Attitude (n=100)

Level of Attitude	Frequency	Percentage (%)
Good	94	94%
Enough	2	2%
Not enough	4	4%
Total	100	100%

Based on table 3, the research results show that as many as 94% of respondents have an attitude in the good category, with 94 respondents accounting for 94% of the total sample. There were 2 respondents (2%) in the sufficient category and 4 respondents (4%) in the insufficirnt category.

DISCUSSION

1. Level of knowledge of nurses as first responders in in-hospital cardiac arrest (IHCA) events

Based on the research results, it was found that the level of knowledge of research respondents regarding in-hospital cardiac arrest (IHCA) was in the good category. The results of this research are in line with research Dhani Irawan et al. (2021). The research concluded that the majority of respondents had good knowledge of implementing BLS (64.2%). In another study conducted by Zamziri et al. (2022), out of 36 research respondents, the results showed that 21 respondents (58.3%) had a good level of knowledge. Research (Karunia Estri et al., 2023) found that 19 people (47.5%) had a good level of knowledge about basic life support.

Increasing a person's knowledge does not have to be obtained through formal education but can also be obtained through non-formal education such as seminars or training. The level of knowledge of nurses is categorized as good because the majority of nurses have undergone BTCLS and ATCLS training. One of the factors that influences knowledge is the source of information. It can be said that wider access to information can help someone learn new things, so that it will increase the level of knowledge of nurses if they are given information regularly. The level of knowledge of nurses will increase as training or seminars are provided regarding basic life support material on a regular basis. So that nurses' knowledge can increase and maintain good knowledge about handling in-hospital cardiac arrest (IHCA).

The majority of respondents in this study have work experience of >21 years (53%). Researchers assume that work experience is another factor that influences a good level of knowledge in this study. The longer someone works, the more knowledge they gain. The work environment is one of the factors that helps a person gain knowledge and experience, either directly or indirectly (Ahmad et al., 2022). A person's experience will certainly provide knowledge and skills for a nurse in dealing with new problems (Juliana, 2019).

2. Level of attitude of nurses as first responders in in-hospital cardiac arrest (IHCA) events

Based on the research results, it shows that the majority of respondents have a good attitude level as first responders in in-hospital cardiac arrest incidents. This is proven by research data that the majority of respondents have a good attitude when dealing with cardiac arrest clients by answering the options agree and strongly agree. The results of this research are in line with research conducted by Dhani Irawan et al. (2021) Research respondents had a positive attitude toward implementing BHD (87.2%). Research conducted (Pangandaheng, 2020) showed that 18 respondents (78.3%) had a good attitude toward carrying out BHD in hospitals.

The results of this study illustrate that there are more female nurses in the ward compared to male nurses. This research is in line with research (Zenitha Victoria et al., 2022), where the number of female respondents was greater than the number of the number of male respondents. This difference in the number of nurses is caused by men's lack of interest in pursuing nursing science, but the proportion of men and women in the work of a nurse is the same.

The research results show that the average length of work for nurses is >21 years (53%), so it can be assumed that length of service can be a factor related to a person's attitude. Increasing the length of service will go hand in hand with the growth of a positive attitude within a person. According to Agus Cahyono et al. (2019) the more experience a person has, the better attitudes they can apply. Nurses who have worked in hospitals for a long time have experience dealing with victims of cardiac arrest in hospitals. With this experience, the nurses will be more alert when they encounter victims.

Apart from the length of service of a nurse, the level of education of a health worker has a significant influence on a nurse's attitude. The formal education of nurses at PKU Muhammadiyah Hospital in Yogyakarta is that the majority have a Diploma 3 or D3 level education. This research is in line with research conducted by Zahara, Jufrizal, and Fikriyanti (2022), which stated that the highest level of education was diploma, with as many as 58 respondents and a percentage of 63.0%. People who undergo formal education are used to thinking logically in dealing with various problems because, in the formal education process, they are taught how to identify, analyze, and find solutions related to a problem. Education has an influence on intelligence, which is in line with a person's thinking power. The higher the level of education of a nurse, the better her knowledge of treating cardiac arrest victims.

Doi: 10.18196/umygrace.v4i1.668

Training, length of work, and education are factors that influence a nurse's knowledge. Apart from what was stated, there are other factors that influence knowledge. In the journal written by Chik et al. (2023), self-confidence, teamwork, availability of tools, and workforce are things that influence the level of knowledge of a nurse in handling arrests. heart in the hospital.

CONCLUSION AND RECOMMENDATION

Conclusion

Based on research conducted at PKU Hospital Muhammadiyah Yogyakarta the following results were obtained nurses' level of knowledge and level of attitude related to in-hospital cardiac arrest is in the category of good.

Recommendation

For PKU Muhammadiyah Yogyakarta Hospital, it is expected to hold training and evaluation of nurses with sufficient knowledge and attitudes and less related to the treatment of in-hospital cardiac arrest, as well as nursing nurses in the hospital who have not undergone BTCLS or ATCLS training aimed at improving nurse knowledge.

For Further Researcher, In the data collection method, it would be better if the researcher met the nurse directly. Researchers can also study related first responders that have not been studied in this research.

REFERENCE

1. 2021_Book Chapter_Metodologi Penelitian Kesehatan. (N.D.).
2. Ahmad, A. J., Mappamiring, & Mustari, N. (2022). Pengaruh Lingkungan Kerja Terhadap Kinerja Pegawai di Dinas Pendidikan dan Kebudayaan Kabupaten Bulukumba. Kajian Ilmiah Mahasiswa Admnistrasi Publik, 3(1), 287–298. https://journal.unismuh.ac.id/index.php/kimap/index
3. American Heart Association (2020). Guidelines For Resuscitation and Emergency Cardiovascular Care.
4. American Red Cross. Basic Life Support for Healthcare Providers Handbook.2015.
5. Cpr, P., & Ecc, D. (2020). Fokus Utama Pedoman American Heart Association 2020 Untuk Cpr Dan Ecc.
6. Dame, R. B., & Kumaat, L. T., 2018, 'Gambaran Tingkat Pengetahuan Perawat Tentang Code Blue System' Jurnal E-Clinic (Ecl), Vol. 6, No. 2, Hh. 162-168
7. Darwati, L. E., Yulianto, I., & Setianingsih. (2019). Tingkat Pengetahuan dan Keikutsertaan Pelatihan CPR Perawat dengan Penanganan Dasar Pasien Henti Jantung berdasarkan Guidelines AHA 2015. Jurnal Gawat Darurat, 1(1), 39–44.
8. Dhani Irawan, D., Sukmaningtyas, W., Novitasari, D., Keperawatan, P., Sarjana, P., Kesehatan, F., Harapan Bangsa, U., Program, A., & Terapan, S. (2021). Hubungan Pengetahuan Dan Sikap Dengan Perilaku Perawat Tim Kode Biru Dalam Pelaksanaan Bantuan Hidup Dasar (Bhd).
9. Ika Safitri, Y., Zenitha Victoria, A., & Dwi Nugroho, K. (2022). Gambaran Kejadian dan Penanganan In-Hospital Cardiac Arrest (IHCA).
10. Ismiroja, Rahmat. Mulyadi. K. M. (2018). Pengalaman Perawat Dalam Penanganan Cardiac Arrest Di Instalasi Gawat Darurat Rsup Prof. Dr. R. D. Kandou Manado (Vol. 6, Issue 2).
11. Juliana. (2019). Gambaran Pengetahuan Perawat Dalam Melakukan Bantuan Hidup Dasar (Bhd) Diruangan Intensive Care Unit (Icu) Rsud Dr. Pirngadi Medan.
12. Karunia Estri, A., Adi Mulyanto, V., Studi Sarjana Keperawatan, P., Panti Rapih Yogyakarta, Stik., Kunci, K., & Hidup Dasar, B. (2023). Levels Of Nurse Knowledge and Skills In Simulation Of Basic Life Support. Cendekia Medika: Jurnal Stikes Al-Ma'arif Baturaja, 8(1).
13. PERKI. (2017). Henti Jantung. https://inaheart.org/henti-jantung/ diperoleh tanggal 22 Oktober 2021
14. Subhan, N., Giwangkencana, G. W., Prihartono, M. A., & Tavianto, D. (2019). Implementasi Early Warning Score pada Kejadian Henti Jantung di Ruang Perawatan Rumah Sakit Dr. Hasan Sadikin Bandung yang Ditangani Tim Code Blue selama Tahun 2017. Jurnal Anestesi Perioperatif, 7(1), 33–41. https://doi.org/10.15851/jap.v7n1.158.
15. Tommy. Pangandaheng. (2020). Hubungan Tingkat Pengetahuan Dengan Sikap Perawat Tentang Penatalaksanaan Bantuan Hidup Dasar.
16. Victoria, A. Z., Ryandini, F. R., Wati, F. A. (2022). Gambaran Pengetahuan Dan Penanganan Perawat Sebagai First Responder Pada Kejadian in Hospital Cardiac Arrest (IHCA). Jurnal Nursing Update-Vol.13 No.4 (2022).
17. Zahara, Jufrizal, Fikriyanti. (2022). Gambaran Pengetahuan Perawat Dalam Melakukan Bantuan Hidup Dasar. In JIM (Issue 4).
18. Zamziri, Maktum. U. (2022). Gambaran Pengetahuan Perawat Dalam Melakukan Bantuan Hidup Dasar (Bhd) Di Rumah Sakit Umum Depati Hamzah Pangkalpinang. Artikel Penelitian Jurnal Keperawatan Sriwijaya, 9.

Doi: 10.18196/umygrace.v4i1.669

Optimization of pH to Bacteriocin Production by *Lactobacillus plantarum* Against *Methicillin-Resistant Staphylococcus aureus*

Naila El Khumaira[1, a)], and Lilis Suryani [2. b)]

[1]Medicine Studies, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Indonesia, 55183
[2]Departement of Microbiology, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183

Email: naila.el.fkik22@umy.ac.id[a)]; lilis.suryani@umy.ac.id[b)]

ABSTRACT

Introduction: *Methicillin-resistant Staphylococcus aureus* (MRSA) is a bacterium that is resistant to beta-lactam antibiotics. MRSA is transmitted through skin/object contact and is often found in health care and the community. MRSA infection has a high mortality and morbidity rate because it causes sepsis, septic shock, respiratory failure, and amputation. *Lactobacillus plantarum* produces bacteriocin. The growth of both gram-positive and gram-negative bacteria can be inhibited by this bacteriocin. **Purpose**: To determine the effect of pH on the activity of bacteriocins produced by *Lactobacillus plantarum* against MRSA. **Method**: This research used quasi-experimental methods. *L.plantarum* was grown in liquid MRS media for 48 hours. Bacteriocin was obtained by centrifuging an MRS broth containing bacteria at a speed of 6000 rpm for 40 minutes. Using the Kirby Bauer method, the liquid formed was tested for its antibacterial effect against MRSA bacteria. Bacteriocins were treated with different pH, namely, 2, 4, 6, 8, and 10. The diameter of the inhibition zone formed during the testing process is measured in millimeters. Data were analyzed using the ANOVA method. **Finding**: The bacteriocin activity produced by Lactobacillus plantarum against MRSA shows an average diameter of the inhibition zone of 3 mm. The optimum broadest spectrum of antibacterial activity was shown in bacteriocins treated with pH 2. **Conclusion**: This study shows the results that pH does not affect the antibacterial activity of bacteriocins produced by *L.plantarum* against MRSA. **Originality/Implication**: The results of this study prove that various pH treatments do not affect the ability of the bacteriocin produced by *L.plantarum* to inhibit/kill MRSA.

Keywords: MRSA, pH, Bacteriosin, *Lactobacillus plantarum*

ABSTRACT

Introduction: *Methicilin resistant Staphylococcus aureus* (MRSA) adalah bakteri yang memiliki resistensi terhadap antibiotik golongan beta laktam. MRSA menular melalui kontak kulit dan permukaan benda, banyak ditemukan di lokasi pelayanan kesehatan dan komunitas. Infeksi MRSA mempunyai angka mortalitas dan morbiditas yang tinggi karena mengakibatkan komplikasi sepsis, syok septic, kegagalan pernapasan, dan amputasi. Bakteri *L. plantarum* menghasilkan bakteriosin. Pertumbuhan bakteri gram positif dan gram negative dapat dihambat oleh bakteriosin. **Purpose**: mengetahui pengaruh pH terhadap aktivitas bakteriosin yang dihasilkan oleh *Lactobacillus plantaru*m terhadap MRSA. **Method**: Penelitian ini menggunakan metode quasi-eksperimental laboratorium. *L. plantarum* ditumbuhkan pada media MRS cair selama 48 jam. Bakteriosin diperoleh dengan cara mensentrifuse MRS cair yang berisi bakteri pada kecepatan 6000 rpm selama 40 menit. Cairan yang terbentuk diuji efek antibakterinya terhadap bakteri MRSA menggunakan metode Kirby Bauer. Bakteriosin diberi perlakuan dengan pH yang berbeda yaitu; 2, 4, 6, 8, dan 10. Diameter zona hambat yang terbentuk selama proses pengujian diukur dengan satuan millimeter. Data dianalisis dengan metode ANOVA. **Finding**: Aktivitas bakteriosin yang dihasilkan *Lactobacilus plantarum* terhadap MRSA menunjukkan rata-rata diameter zona inhibisi sebesar 3 mm. Spektrum aktivitas antibakteri terluas menunjukkan pada bakteriosin dengan perlakuan pH 2. **Conclusion**: Studi ini menunjukkan hasil bahwa pH tidak mempengaruhi aktivitas antibakteri bakteriosin yang dihasilkan *L. plantarum* terhadap MRSA. **Originality/Implication:** Hasil penelitian ini membuktikan bahwa perlakuan pH dengan berbagai variasi tidak mempengaruhi kemampuan bakteriosin yang dihasilkan *L. plantarum* dalam menghambat/membunuh MRSA.

Keywords: MRSA, pH, Bacteriosin, *Lactobacillus plantarum*

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a type of *S. aureus* bacteria that is resistant to various antibiotics, also called multidrug-resistant microorganisms (MDRM) (Otto, 2013). MRSA infections that are often found in hospitals or

 Doi: 10.18196/umygrace.v4i1.669

health facilities are known as HA-MRSA (Hibbitts and O'Leary, 2018). Meanwhile, CA-MRSA comes from the community or general public, while LA-MRSA is found in livestock environments (Peacock and Paterson, 2015; Siddiqui and Koirala, 2021).

According to recent research published in the Lancet report, MRSA caused more than 100,000 deaths in 2019 (Murray et al., 2022). Symptoms of HA-MRSA and CA-MRSA infections include cellulitis and abscesses, or both, where these skin infections can spread to deeper tissues (Green et al., 2012). The antibiotics usually used as the first choice for treating MRSA infections are vancomycin or daptomycin, with alternative options such as telavancin, ceftaroline, and linezolid (Choo and Chambers, 2016). However, there have been reports of vancomycin-resistant MRSA strains, known as *vancomycin-resistant Staphylococcus aureus* (VRSA and VISA), with increased minimum inhibitory concentration (MIC) indicating reduced sensitivity. This limits future therapeutic options, so alternative treatments are needed (Kali, 2015).

As creatures created by God who are gifted with reason, humans must be able to develop new ideas. One example is characterizing bacteriocins as an alternative to treat antibiotic-resistant MRSA. This is in line with the word of Allah in QS. Al-Baqarah verse 26: "Indeed, Allah is not reluctant to make an example of a mosquito or something lower than that..."

Proteins called bacteriocins are synthesized by lactic acid bacteria (LAB) and can inhibit microbes. Characteristics of bacteriocins include sensitivity to proteolytic enzymes, resistance to high temperatures, and stability at acidic or neutral pH (Widodo et al., 2018). The bacteriocins' mechanism of action includes binding to lipid I and lipid II, which are crucial precursors in the bacterial cell wall synthesis process, to inhibit cell wall synthesis. (Gupta and Pandey, 2019). Bacteriocins have greater efficacy than antibiotics (Morisset, 2002) and also have a synergistic effect and are more economical compared to expensive antibiotic production (Mathur, 2017).

The purpose of this study was to investigate the effectiveness of *L. plantarum*-produced bacteriocin in restraining the growth of MRSA bacteria under different pH conditions. Additionally, the goal was to identify the most suitable pH for the bacteriocin to effectively inhibit or eradicate MRSA bacteria.

LITERATURE REVIEW

(1) *Methicillin resistant Staphylococcus aureus* (MRSA)
Methicillin-resistant Staphylococcus aureus is a bacteria that is resistant to antibiotics. Currently, these resistant bacterial strains have been recognized as a major problem in many countries, both developing and developed (Amalia et al., 2017). The bacteria develop resistance due to the presence of the mecA gene, which encodes Penicillin Binding Protein 2a (PBP2a). This protein has a reduced affinity for beta-lactam antibiotics, resulting in methicillin resistance. (Susanti et al., 2020). These bacteria can cause various conditions such as bacteremia, osteomyelitis, endocarditis, skin infections, pneumonia, and soft tissue infections (Jayanthi et al., 2020).

(2) *L. plantarum*
Lactobacillus plantarum is a non-motile, rod-shaped gram-positive bacterium (Zheng et al., 2020). This bacterium has negative catalase properties, can live in aerobic or facultative anaerobic conditions, quickly digests protein, and is acid tolerant. *L. plantarum* usually forms short stems under optimal growing conditions but tends to elongate under less favorable conditions. These bacteria can produce bacteriocins, namely polypeptides or bactericidal protein compounds (James et al., 1992).

(3) Bacteriosins
Bacteriocins are a group of antimicrobial peptides that function as agents inhibiting bacterial growth. Bacteriocins are synthesized by ribosomes and undergo post-translational modification to become the active form (Mokoena, 2017). Various types of bacteriocins have been identified, each operating through distinct mechanisms, including creating pores to induce cytoplasmic membrane permeability, hindering cell wall biosynthesis, and interfering with metabolic pathways. (Hols et al., 2019).

The use of bacteriocins as antimicrobial agents has been proven safe because they can be broken down by protease enzymes and do not cause resistance (Chen et al., 2022). Bacteriocins can work by preventing colonization or killing pathogenic bacteria that enter the digestive tract (Anjana & Tiwari, 2022). Bacteriocin-producing probiotic strains can prevent pathogenic bacteria from adhering to the intestinal wall by colonizing the intestines. They achieve this through competition, clearing out their own niche, and segregating themselves spatially. (Heilbronner et al., 2021).

(4) Bacterial Sensitivity Test
The method for testing antibacterial activity can be carried out through diffusion, which is generally known as the Kirby and Bauer test. The principle of this method involves the diffusion of antibacterial compounds onto paper discs which are then placed on agar media that has been inoculated with bacteria and incubated for 18-24 hours at 35°C. The clear zone that forms around the disc indicates an inhibitory area for bacterial growth (Balaouri et al., 2016).

METHOD

A. Study Design
This study is a laboratory experiment carried out between May and June 2024 at the Microbiology Laboratory of the Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta. The research received approval from the Health

Doi: 10.18196/umygrace.v4i1.669

Research Ethics Committee of FKIK UMY under the number 046/EC-EXEM-KEPK FKIK UMY/V/2024.

B. Samples

The research sample comprised Lactobacillus plantarum obtained from the PAU UGM laboratory located on Jl. Teknika Utara, Caturtunggal, Depok, Sleman Regency, Yogyakarta Special Region. The test bacteria employed were local isolates of Methicillin-resistant Staphylococcus aureus (MRSA) sourced from the Microbiology Laboratory of the Faculty of Medicine UGM.

C. Tools and Materials

The tools used in this research include petri dishes (Iwaki Pyrex), tube needles, glass slides, test tubes, Erlenmeyer tubes (Iwaki Pyrex), beakers (Iwaki Pyrex), microscopes, incubators, biosafety cabinets, Bunsen cupboards. cooler, centrifuge, litmus paper, and caliper.

The materials used in this research include: *Lactobacillus plantarum* isolate, De Man Rogosa and Sharpe (MRS) agar medium, De Man Rogosa and Sharpe (MRS) broth medium, distilled water, crystal violet, iodine, 95% ethanol, safranin, buffer phosphate, NaOH, HCl, Mueller Hinton Agar (MHA), and Trypticase Soy Agar (TSA).

D. Research Procedure

1) *Lactobacillus plantarum* Bacterial Culture Rejuvenation

Lactobacillus plantarum culture was renewed through subculture on MRS agar media using the Streak Plate method. Bacterial colonies were inoculated into MRS agar media using a sterile loop by streaking. The MRS agar medium was then incubated at 37°C for 48 hours under anaerobic conditions. The colonies that grow are then identified using gram staining.

2) Identification of *Lactobacillus plantarum* bacteria using Gram staining

The method for identifying bacteria began with the application of a single drop of crystal violet to the smear containing a 24-hour-old *Lactobacillus plantarum* isolate. After one minute, the preparation was rinsed with distilled water. Subsequently, a drop of iodine was applied, left in place for 2 minutes, and then washed with distilled water. The preparations were washed with 95% ethanol and then rinsed with running water. Following this, the isolate was stained with safranin and rinsed with running water. Finally, the preparations were air-dried at room temperature and examined under a microscope at 1000x magnification.

3) Isolation of Bacteriocins Produced by *Lactobacillus plantarum*

Lactobacillus plantarum bacterial isolates that had grown on MRS agar for 48 hours were inoculated into 10 ml of MRS broth media and incubated for 48 hours at 37°C under anaerobic conditions. After that, the culture was centrifuged at 6000 rpm for 30 minutes twice. 5 ml of supernatant fluid was taken and put into an Eppendorf tube. This liquid is used as a source of bacteriocin and is ready to be tested for its antibacterial activity.

4) pH treatment of bacteriocins

A total of 5 ml of bacteriocin was divided into 5 groups, each group was given a different pH treatment, namely 2, 4, 6, 8, and 10. Each pH treatment was repeated twice. pH treatment was carried out by adding bacteriocin to an Eppendorf tube with a volume of 1.25 ml for each treatment. Then, the bacteriocin is dropped with 1N HCl or NaOH according to the desired pH. After that, the antibacterial activity of bacteriocins against *Methicillin-resistant Staphylococcus aureus* (MRSA) was tested using the Kirby Bauer method.

5) *Methicillin-resistant Staphylococcus aureus* (MRSA) Test Bacteria Preparation

MRSA test bacteria were inoculated into TSA media and incubated at 37°C for 24 hours under aerobic conditions. To prepare the MRSA inoculum, several MRSA colonies were taken from the TSA medium using a loop and transferred into 2 ml of BHI medium. Inoculum turbidity was adjusted to the 0.5% McFarland standard which corresponds to 1.5 × 10^8 CFU/mL.

6) Antibacterial Testing of Bacteriocins against *Methicillin-resistant Staphylococcus aureus* (MRSA) using the Kirby Bauer Method

MRSA test bacteria with a concentration of 1.5 × 10^8 CFU/mL were grown on Mueller Hinton Agar media using sterile cotton swabs. The test bacterial suspension is applied to the MHA media using a sterile cotton swab, and then allowed to absorb into the media for 5-15 minutes. A 20 µl bacteriocin supernatant suspension, treated with various pH, is added to a paper disc. The disc is then placed on MHA media inoculated with the test bacteria and incubated at 37°C for 24 hours under aerobic conditions. The diameter of the inhibition zone formed is then measured using a caliper in millimeters. The size of the inhibition zone is used to evaluate the antimicrobial activity of bacteriocins from *Lactobacillus plantarum* against MRSA bacteria.

7) Data Analysis

 Doi: 10.18196/umygrace.v4i1.669

Data on the diameter of the inhibition zone from each treatment were analyzed using the ANOVA test to evaluate the effect of pH treatment on the activity of bacteriocins produced by *Lactobacillus plantarum* against MRSA.

RESULT AND DISCUSSION

(1) Results

Examination of the activity of bacteriocins produced by *Lactobacillus plantarum* against MRSA was carried out through identification of *Lactobacillus plantarum* and antibacterial tests. Identification using Gram staining shows that *Lactobacillus plantarum* is a gram-positive bacterium with a rod cell shape (Figure 1). On MRS agar media, the bacterial colonies appear round with a light white-brown color.

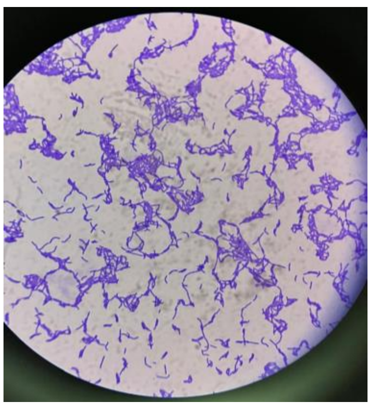


Figure 1. Bacterial cell shape of *Lactobacillus plantarum* by Gram stain

Diagram 1 shows the results of examining the activity of bacteriocins produced by *Lactobacillus plantarum* against *Methicillin-resistant Staphylococcus aureus* (MRSA) with various heating temperature treatments. The average diameter of the inhibition zone is 3 millimeters. This research shows that the bacteriocin produced by the bacteria *Lactobacillus plantarum* has activity against MRSA bacteria.

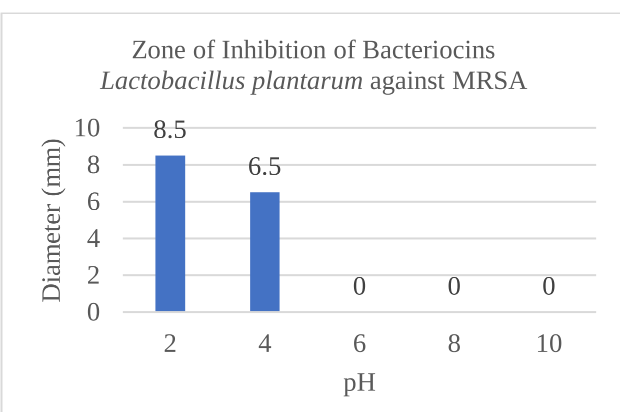


Diagram 1. Diameter of inhibition zone of bacteriocin produced by *Lactobacillus plantarum* against MRSA

The diameter of the bacteriocin inhibition zone with pH 2 treatment showed the highest antibacterial activity, namely 8.5 mm. at pH 6, 8, and 10 bacteriocins did not show antibacterial activity. From these data, information was obtained that pH 2 is optimal for the antibacterial activity of bacteriocins against MRSA. The results of the ANOVA test analysis obtained P= 0.072. The results of these statistical tests prove that temperature does not affect the activity of *Lactobacillus plantarum* bacteriocin against MRSA.

(2) Discussion

This research proves that pH does not affect the activity of bacteriocins produced by *L.plantarum* against MRSA and this research also proves that bacteriocins from *L.plantarum* have antibacterial power against *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria. The optimal pH for the antibacterial activity of bacteriocins produced by *L. plantarum* is pH 2 with an average inhibition zone diameter of 8.5 mm. The antibacterial inhibition zone's activity can be categorized into four groups: low activity (<5 mm), moderate (5-10 mm), high (>10-20 mm), and very high (>20-30 mm) (Ismail et al., 2017). The greater the clear zone on the MHA media, the greater the antibacterial activity against pathogens. From the results of this treatment, it was found that the antibacterial activity of the bacteriocin produced by the *L. plantarum* isolate was in the moderate group against *Methicillin-resistant Staphylococcus aureus (MRSA)*. Meanwhile, the average value of the diameter of the inhibition zone formed by bacteriocins in all pH treatments is 3 mm, the size of this diameter falls into the group of weak antibacterial activity with a diameter range of <5 mm.

Lactic acid bacteria from various genera such as *Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus acidophilus*, and *Lactobacillus plantarum* have the capability to produce bacteriocins (Scallan et al., 2011). Antibacterial activity generally increases under acidic pH conditions because low pH increases the interaction of bacteriocins with receptors on the membrane. Bacteriocin molecules attach more effectively to cell walls in acidic conditions, thereby increasing their bactericidal potential (Parada et al., 2007).

Bacteriocin activity can differ at different pH levels due to intramolecular electrostatic interactions resulting from the separation of amino and carboxyl groups at extreme pH, leading to protein denaturation (Yi et al., 2016; Jiang et al., 2017). Bacteriocins produced from *L. plantarum* isolates showed much higher antimicrobial effectiveness at pH 2 and 4 than at pH 6, 8, and 10. This may be related to the high tolerance of lactic acid bacteria (LAB) to low pH conditions (Chartier et al., 2014) . These findings are in line with research by Malini M and Savitha J (2012), who noted that pure bacteriocins from LAB showed optimal antibacterial activity against Staphylococcus aureus at low pH, especially at pH 2 and 4. In

 Doi: 10.18196/umygrace.v4i1.669

contrast, bacteriocin extract diluted at pH 10 showed very low antimicrobial activity against LAB (Kurniawan, 2012).

Apart from being influenced by pH and temperature, the antibacterial activity of bacteriocins is also influenced by the duration of incubation time. Research shows that the antibacterial activity of bacteriocins reaches the highest level in lactic acid bacteria with incubation for 14 hours at a temperature of 27°C and pH 6 (Marwati et al., 2018).

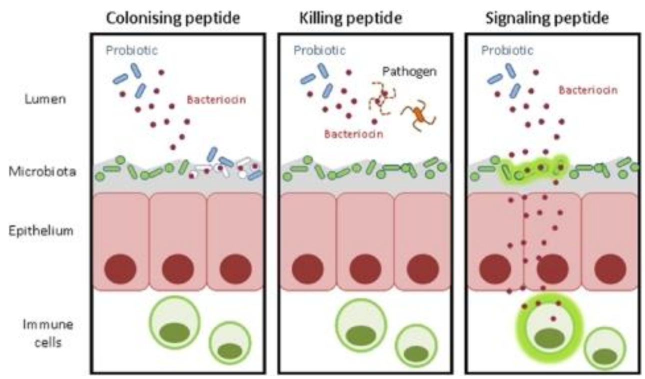


Figure 2. Mechanism of bacteriocins in killing bacteria (Dobson et al., 2011)

Figure 2 illustrates the mechanism of bacteriocins in killing bacteria, where bacteriocins act as colonizing peptides, killing peptides, or signaling. As an antimicrobial peptide compound, bacteriocins help producer bacteria compete with other bacterial strains, thereby supporting the survival of producer bacteria in the digestive environment. An example of this competition is seen in E. coli strains that produce colicin, which can survive better than bacteria that do not produce colicin (Gillor et al., 2009). Bacteriocins also function as peptides that kill or inhibit the growth of pathogenic bacteria that can harm the host body. Bacteriocin producers from gram-negative bacteria have greater potential in this function compared to producers from gram-positive bacteria, because pathogenic bacteria are generally gram-negative bacteria (Kirkup, 2006). In addition, bacteriocins can act as signaling agents in multicellular level functional mechanisms, such as immune responses. At high concentrations, bacteriocins function as pathogen inhibitors, while at low concentrations, bacteriocins act more as signal sending agents (Fajardo and Martinez, 2008).

CONCLUSION AND RECOMMENDATION

The bacteriocin produced by *L. plantarum* has antibacterial activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria. This study shows the results that pH does not affect the antibacterial activity of bacteriocins produced by *L. plantarum* against MRSA. The optimal pH to obtain L. plantarum bacteriocin activity is pH 2, with an inhibition zone diameter of 8.5 mm.

Researchers propose further research regarding the antibacterial activity of bacteriocins, especially against various bacteria that are resistant to various antibiotics.

REFERENCE

1. Amalia, A., Sari, I., & Nursanty, R. (2017). Aktivitas antibakteri ekstrak etil asetat daun sembung (*Blumea balsamifera (L.) DC.) terhadap pertumbuhan bakteri methicillin resistant Staphylococcus aureus (MRSA). Prosiding Biotik,* *5*(1). http://dx.doi.org/10.22373/pbio.v5i1.2160

2. Anjana, & Tiwari, S. K. (2022). Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. *Frontiers in Cellular and Infection Microbiology, 12*,851140. https://doi.org/10.3389/fcimb.2022.851140

3. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*,*6*(2),71–79. https://doi.org/10.1016/j.jpha.2015.11.005

4. Chartier, M., Gibernau, M., & Renner, S. S. (2014). The evolution of pollinator-plant interaction types in the Araceae. *Evolution, 68*(5), 1533–1543. https://doi.org/10.1111/evo.12318
5. Chen, J., Pang, H., Wang, L., Ma, C., Wu, G., Liu, Y., Guan, Y., Zhang, M., Qin, G., & Tan, Z. (2022). Bacteriocin-producing lactic acid bacteria strains with antimicrobial activity screened from Bamei pig feces. *Foods, 11*(5), 709. https://doi.org/10.3390/foods11050709/s1

6. Choo, E. J., & Chambers, H. F. (2016). Treatment of *methicillin-resistant Staphylococcus aureus* bacteremia. *Infection and Chemotherapy, 48*(4), 267–273. Korean Society of Infectious Diseases, Korean Society for Chemotherapy. https://doi.org/10.3947/ic.2016.48.4.267
7. Fajardo, A., & Martinez, J. L. (2008). Antibiotics as signals that trigger specific bacterial responses. *Current Opinion in Microbiology, 11*(2), 161–167. https://doi.org/10.1016/j.mib.2008.02.006

8. Gillor, O., Giladi, I., & Riley, M. A. (2009). Persistence of colicinogenic *Escherichia coli* in the mouse gastrointestinal tract. *BMC Microbiology, 9*, 165. https://doi.org/10.1186/1471-2180-9-165

9. Green, B. N., Johnson, C. D., Egan, J. T., Rosenthal, M., Griffith, E. A., & Evans, M. W. (2012). *Methicillin-resistant Staphylococcus aureus*: An overview for manual therapists. *Journal of Chiropractic Medicine, 11*, 64–76. https://doi.org/10.1016/j.jcm.2011.12.001

Doi: 10.18196/umygrace.v4i1.669

10. Gupta, V. K., & Pandey, A. (Eds.). (2019). *New and future developments in microbial biotechnology and bioengineering*. United Kingdom: Elsevier.
11. Heilbronner, S., Krismer, B., Brötz-Oesterhelt, H., & Peschel, A. (2021). The microbiome-shaping roles of bacteriocins. *Nature Reviews Microbiology, 19*(11), 726–739. https://doi.org/10.1038/s41579-021-00569-w
12. Hibbitts, A., & O'Leary, C. (2018). Emerging nanomedicine therapies to counter the rise of methicillin-resistant *Staphylococcus aureus*. *Journal of Materials, 11*(2), 321. https://doi.org/10.3390%2Fma11020321
13. Hols, P., Ledesma-García, L., Gabant, P., & Mignolet, J. (2019). Mobilization of microbiota commensals and their bacteriocins for therapeutics. *Trends in Microbiology, 27*(8), 690–702. https://doi.org/10.1016/j.tim.2019.03.007
14. Ismail, Y. S., Yulvizar, C., & Putriani, P. (2017). Isolasi, karakterisasi, dan uji aktivitas antimikroba bakteri asam laktat dari fermentasi biji kakao (*Theobroma cacao* L.). *Jurnal Bioleuser*, *1*(2). https://jurnal.usk.ac.id/bioleuser/article/view/9072
15. James, R., Lazdunski, C., & Pattus, F. (1992). *Bacteriocins, microcins, and lantibiotics*. Springer-Verlag.
16. Jayanthi, A. A. I., Tarini, N. M. A., & Praharsini, I. G. A. A. (2020). *Staphylococcus aureus* sebagai agen penyebab infeksi pada kasus erisipelas kruris dekstra dengan liken simpleks kronikus. *Intisari Sains Medis, 11*(3), 1482–1491.
17. Jiang, H., Zou, J., Cheng, H., Fang, J., & Huang, G. (2017). Purification, characterization, and mode of action of pentocin JL-1, a novel bacteriocin isolated from *Lactobacillus pentosus*, against drug-resistant *Staphylococcus aureus*. *BioMed Research International, 2017*. https://doi.org/10.1155/2017/7657196
18. Kali, A. (2015). Antibiotics and bioactive natural products in treatment of *methicillin-resistant Staphylococcus aureus*: A brief review. *Pharmacognosy Reviews, 9*(17), 29–34. Medknow Publications. https://dx.doi.org/10.4103/0973-7847.156329
19. Kirkup, B. C. Jr. (2006). Bacteriocins as oral and gastrointestinal antibiotics: Theoretical considerations, applied research, and practical applications. *Current Medicinal Chemistry, 13*(28), 3335–3350. https://doi.org/10.2174/092986706778773068
20. Kurniawan, F. A. M. (2012). Analisis ketahanan bakteriosin dari *Lactobacillus plantarum* 1a5, 1b1, 2b2, dan 2c12 pada pH asam dalam menghambat aktivitas bakteri patogen (Skripsi, Institut Pertanian Bogor). Bogor: Institut Pertanian Bogor.
21. Malini, M., & Savitha, J. (2012). Detection of heat stable bacteriocin from *Lactobacillus acidophilus* NCIM5426 by liquid chromatography/mass spectrometry. *Indian Journal of Science and Technology, 5*(3). https://dx.doi.org/10.17485/ijst/2012/v5i3.23
22. Marwati, T., Cahyaningrum, N., Widodo, S., Januarsyah, T., & Purwoko. (2018). Inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 toward *L. monocytogenes, S. thypimurium* and *E. coli*. *IOP Conference Series: Earth and Environmental Science*, *102*, 012091. https://doi.org/10.1088/1755-1315/102/1/012091
23. Mathur, H., Bidang, D., Rea, M. C., Cotter, P. D., Hill, C., & Ross, R. P. (2017). Bacteriocin-antimicrobial synergy: A medical and food perspective. *Frontiers in Microbiology, 8*, 1205. https://doi.org/10.3389/fmicb.2017.01205
24. Mokoena, M. P. (2017). Lactic acid bacteria and their bacteriocins: Classification, biosynthesis, and applications against uropathogens: A mini review. *Molecules, 22*(8), 1255. https://doi.org/10.3390/molecules22081255
25. Morisset, D., & Frère, J. (2002). Heterologous expression of bacteriocins using the mesentericin Y105 dedicated transport system by *Leuconostoc mesenteroides. Biokimia, 84*(5–6), 569–576. https://doi.org/10.1016/s0300-9084(02)01413-x
26. Murray, C. J., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
27. Otto, M. (2013). Community-associated MRSA: What makes them special? *International Journal of Medical Microbiology, 303*(6–7), 324–330. https://doi.org/10.1016/j.ijmm.2013.02.007
28. Parada, J. L., Caron, C. R., Medeiros, A. B. P., & Ricardo, C. (2007). Bacteriocin from lactic acid bacteria: Purification, properties, and use as biopreservatives. *Brazilian Journal of Biology and Technology, 50*, 521–542. http://dx.doi.org/10.1590/S1516-89132007000300018
29. Peacock, S. J., & Paterson, G. K. (2015). Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annual Review of Biochemistry, 84*, 577–601. https://doi.org/10.1146/annurev-biochem-060614-034516

Doi: 10.18196/umygrace.v4i1.669

30. Scallan, E., et al. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*. https://doi.org/10.3201%2Feid1701.P11101

31. Siddiqui, A. H., & Koirala, J. (2023). *Methicillin-resistant Staphylococcus aureus*. In *StatPearls* [Internet]. StatPearls Publishing. Available from https://www.ncbi.nlm.nih.gov/books/NBK482221/

32. Susanti, M. A., Mahardhika, G. S., Rujito, L., Darmawan, A. B., & Anjarwati, D. U. (2020). The examination of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA) and inappropriate antibiotic use by healthcare workers and communities in Banyumas. *Jurnal Kedokteran dan Kesehatan Indonesia, 11*(3), 257–265. https://doi.org/10.20885/JKKI.Vol11.Iss3.art7

33. Widodo, Wahyuningsih, T. D., Nurrocmad, A., Wahuni, E., Taufiq, T., Anindita, N. S., Lestari, S., Harsita, O., Sukarno, A., & Handaka, R. (2018). *Bakteri asam laktat strain lokal*. UGM Press.

34. Yi, L., Dang, Y., Wu, J., Zhang, L., Liu, X., Liu, B., & Lu, X. (2016). Purification and characterization of a novel bacteriocin produced by *Lactobacillus crustorum* MN047 isolated from koumiss from Xinjiang, China. *Journal of Dairy Science, 99*(9), 7002–7015. https://doi.org/10.3168/jds.2016-11166

35. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S (2020) A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol 70:2782–2858. https://doi.org/10.1099/ijsem.0.004107

36. Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'Toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology, 70*, 2782–2858. https://doi.org/10.1099/ijsem.0.0041