

PAPER • OPEN ACCESS

Bacteriocin from Donggala cow's milk againsts *Salmonella typhi*

To cite this article: A Nurindasari *et al* 2019 *J. Phys.: Conf. Ser.* **1277** 012008

View the [article online](#) for updates and enhancements.

You may also like

- [Characteristics of antibacterial activity stability of crude bacteriocin *Pediococcus acidilactici* BK01](#)
S Melia, I Juliyarsi, Y F Kurnia et al.
- [Inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 toward *L. monocytogenes*, *S. thymurium* and *E. coli*](#)
T Marwati, N Cahyaningrum, S Widodo et al.
- [The Growth and Bacteriocin Productions of *Enterococcus Faecium* Cultured in Aerobic and Anaerobic Conditions](#)
Muhamad Ali, Melinda Sanggu Artha, Muhamad Aidil Fadjar Suryadi et al.



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

Bacteriocin from Donggala cow's milk againsts *Salmonella typhi*

A Nurindasari*, S Bukang, M Ananda, I N Suwastika

Department of Biology, Faculty of Sciences, Tadulako University, Jl. Soekarno-Hatta
Km. 9, Palu 94118, Indonesia

*Corresponding Author : Andinnurindahsari@gmail.com

Abstract. Bacteriocin is a protein compound produced by lactic acid bacteria (LAB) and able to inhibit the growth of pathogenic bacteria. Bacteriocin was isolated from donggala cow's milk (endemic cow of central sulawesi) which is not commercialized. This study was the first report conducted in central sulawesi. The aim of this study was to determine the ability of bacteriocin inhibiting *Salmonella typhi*. The experiments were selection of antimicrobial LAB, activity and stability (temperature and pH) of crude extract of bacteriocin using well diffusion method. There were 9 isolates of LAB and characterized by a clear zone around the colony on MRSA + CaCO₃ 1% media. The result showed that was one isolate (MA 9) having inhibitory activity against *S. typhi* at pH 5 (acid) was 1,3mm and pH 7 (neutral) was 3,17mm. Optimum bacteriocin inhibits *S. typhi* at 37°C and pH 7 (inhibitory activity against *S. typhi* was 2,58mm). Bacteriocin has a potential as an alternative natural preservative.

1. Introduction

Bacteriocin is an extracellular product in the form of protein produced by Lactic Acid Bacteria (LAB) and very beneficial for humans because it can prevent the decaying of food by inhibiting the growth of pathogenic bacteria. The preparation procedures of food that involve heating is one of the bacteriocins' advantages. It is utilized as natural preservatives because bacteriocins are stable in the heating process up to 1000°C [1]. Bacteriocin is generally produced by LAB which is a group of gram-positive bacteria that is safe for the human body because it can be degraded by proteolytic enzymes in the digestive system [2].

Lactic acid bacteria are able to produce antibacterial compounds in the form of lactic acid, CO₂, ethanol, acetaldehyde, diasetil and other compounds which are the result of hydrolysis of glucose and being able to extend the shelf life of its main products of food products [3].

Donggala cows are widely distributed, especially in central Sulawesi, so they are often also called "Sapi Wani". This cow has the ability to adapt easily in hot and dry conditions, that is a great demand by the public because it is easy to maintain. In addition, this local cow kept as meat-producing cows [4].

The absence of research on isolation and production of bacteriocin from local cow milk in Central Sulawesi shows that it is important to conduct research on the potential of LAB isolated from fresh cow's milk in producing bacteriocin which is useful for inhibiting spoilage bacteria and pathogens so that it can be applied as a natural preservative that can replace the presence of chemistry preservatives and it is very beneficial for human health especially in the food sector.



2. Methods

2.1. Milk sampling and Isolation of Lactic Acid Bacteria

Sampling of fresh milk was directly in aseptic conditions at the UPTD Sidera, Sigi Biromaru, Central Sulawesi. 1 ml of fresh milk was put into 9 ml of NaCl 0.9% and inoculated with MRSA (de Man Rogosa Sharpe Agar) + 1% CaCO₃ then incubated at 37°C. samples that form clear zones on the media were purified. Measurement of media pH was done before and after incubation.

2.2. Purification of Lactic Acid Bacteria

Colonies that have been obtained were sub-cultured in Lactose Broth and incubated for 24 hours at 37°C. 1 ml of the samples was put into a new MRSA + 1% CaCO₃ using Spread Plate method and incubated for 48 hours at 37°C. After that, the colonies that form the clear zone were inoculated in MRSA+ 1% CaCO₃ using the quadrant line method to obtain pure culture. The colonies that are separated and form clear zones were scratched on the media as a culture stock and incubated for 24 hours 37°C.

2.3. Characterization of Lactid Acid Bacteria

Macroscopic characterization of samples were carried out by observing the characteristics of the colony including size, color, shape and edges. Then for the catalase assay, 1 ose was applied to a sterile object glass and dripped with 3% H₂O₂ solution. Negative catalase was indicated by the absence of foam formed after being left for 1 minute. Characterization was also carried out by gram staining for the morphology of BAL microscopically.

2.4. Selection of Antimikrobial from LAB

Antimicrobial from LAB against *Salmonella typhi* was tested by a well diffusion method. The suspension of indicator bacteria was inoculated into LB agar. 50 µl were put into the then incubated 37°C for 24 hours. The form of clear zone were measured. As a positive control using chloramphenicol.

2.5. Antimikrobial Activity Assay of Bacteriosin

LAB that have antimicrobial bacteriocin activity were tested by the well diffusion method. 50 µl of supernatant was neutralized (pH 7.0) by 1M NaOH and put into the well which contained indicator bacteria and then incubated at 37°C for 24 hours. Antimicrobial activity was determined from the formed clear zone.

2.6. Temperature and pH Assay

The effect of temperature on bacteriocin activity was carried out at temperatures of 50, 80, and 100° C (for 10 minutes). Crude extract bacteriocin (optimum pH) were given heat shock treatment at 50, 80, 100°C for 10 minutes. Bacteriocin testing of various pH were also carried out by the well diffusion method. Bacteriocin solution was treated with pH 4, 7 and 9 with the addition of 0.1 M HCl for acidic pH and 0.1 M NaOH for alkaline pH. Furthermore, the inhibitory activity was tested against pathogenic bacteria incubated at 37°C.

3. Result and Discussion

The results of LAB isolation showed that there were 9 bacterial isolates. LAB were characterized by clear zone of bacterial colonies (Figure 1). Then 5 isolates of bacteria were able to grow well and continued with characteristif of macro and micro, catalase assay and gram staining (Table 1). LAB is a group of bacteria that non-producing catalase to convert hydrogen peroxide (negative catalase) [5].

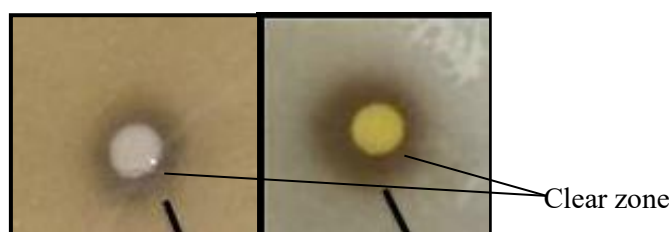


Figure 1. Isolates of lactic acid bacteria that show clear zone in MRSA + CaCO₃ 1%

Table 1. Characteristics of five selected lactic acid bacteria

No.	Isolate	Gram Type	Cell Size (μ)	Shape (micro)	Shape (macro)	Edge	Pigmentation	Catalase Assay
1	I ₂	+	8	Bacill	Circular	Entire	White	-
2	I ₄	+	8-10	Bacill	Circular	Entire	White	-
3	I ₆	+	5-8	Bacill	Circular	Entire	White to yellow	-
4	I ₇	+	7-10	Bacill	Circular	Entire	White to yellow	-
5	I ₉	+	5-7	Bacill	Circular	Entire	White	-

Characterization of five isolates showed that the same character as the group of lactic acid bacteria. This is similar to the result of LAB isolated from fermented food showed the characteristic of rod-shaped cells and was a group of gram-positive bacteria, negative catalase and able to produce anti-bacterial compounds [6].

The antimicrobial activity produced by LAB can be identified by the presence of LAB supernatant assay against pathogen (*S. thypi*) using well diffusion method. The five isolates were centrifuged to obtain cell-free supernatants. The pH measurement was carried out before the supernatant was inserted into the well as a presumption of the initial activity. The results of the measurement of pH was pH 5 which indicates the presence of organic acids that have the potential as antibacterial.

There was one of the five isolates that can inhibit pathogenic bacteria i.e I₉ and able to inhibit *S. thypi* by 1.3 mm of clear zone (Figure 2). The clear zone indicates that I₉ were capable of producing antimicrobials that can inhibit pathogenic bacteria. Organic acids such as lactic acid, hydrogen peroxide, diacetyl, acetaldehyde, reuterine, amino acids and bacteriocins are compounds produced by LAB [7]

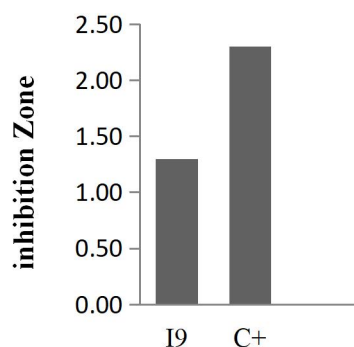


Figure 2. Antimicrobial activity of I₉ against *S. typhi*

Supernatant of I₉ was previously able to inhibit *S. typhi* at pH 5 then neutralized the pH (pH 7) using 1M NaOH. The purpose of giving NaOH was to eliminate the effects of organic acids produced by these bacteria so that the inhibition that occurs due to bacteriocin activity [8]. Isolate I₉ was able to inhibit *S. typhi* by 3.17 mm (Figure 3).

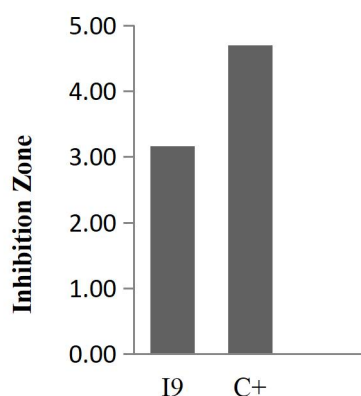


Figure 3. Inhibition zone of MA 9 isolate at pH 7 against *S. typhi*

The clear zone formed after neutralization using NaOH due to inhibitory activity which was not from organic acids. Bacteriocin was able to bind cell surface receptors by entering into target cells by forming pores, inhibiting peptidoglycan synthesis, and also cellular DNA degradation because bacteriocins are low molecular weight proteins generally <10 kDa.

Bacteriocin produced by LAB can inhibit several pathogenic bacteria including gram negative bacteria. This was because bacteriocins generally attack the target cell wall by forming pores so that it was easier to inhibit the growth of thinner cell wall of gram negative bacteria (10-15nm) compared to thicker cell wall of gram-positive bacteria (15-23nm).

The crude extract of bacteriocin were tested for stability against temperature and pH in inhibiting *S. typhi*. The bacteriocin loses its ability after being treated with a temperature of 50, 80, 100 and only shows activity at pH 7. LAB was able to produce bacteriocin if supported by environmental factors such as pH and optimum temperature. Bacteriocin had activity at different pH, where some optimum bacteriocin works at pH 7.

Table 2. Results of stability assay of bacteriocin against *S.typhi*

No.	Supernatant of LAB	Inhibition					
		pH (mm)			Suhu (mm)		
		4	7	9	50	80	100
1.	I ₉	-	2,58	-	-	-	-

4. Conclusion

The crude extract of bacteriocin produced by I₉ isolates from Donggala cow milk has the potential as a natural preservative due to the highest activity (3.17mm of inhibition zone) that occurs at neutral pH (pH 7).

Acknowledgements

The author would like to thank the Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University for facilitating this research.

References

- [1] Muhamed S, Tayeb I, Hourla O H, Heba N., and Salima A 2012 Production an characterization of bacteriocin of *Lactobacillus plantarum* F12 with inhibitory activity against *Listeria monocytogenes*. *The Online Journal of Science and Technology* **2**(1) pp. 55–1.
- [2] Abubakar, and Muhammad A 2015 Pengaruh suhu produksi terhadap aktivitas ekstrak kasar bakteriosin dari berbagai galur. *Buletin Peternakan*. **39**(3) pp. 189–98.
- [3] Tahara T, Oshimura M, Umezawa C, and Kanatani K 1996 Isolation, partial characterization, and mode of action of acidocin J1132, a two-component bacteriocin produced by *Lactobacillus acidophilus* JCM 1132 *Applied and Environmental Microbiology* **62**(3) pp. 892–7.
- [4] Muh H H 2016 Komposisi Asam Lemak Neutral Lipid of Muscle dan Intermuscular Adipose Tissue Lipid Antara Sapi Weaners dan Mutures *Jurnal Agrisains* **17**(1) pp 1-7.
- [5] Rafika S, Cesilia A, Maksun R, Amarila M 2011 Skrining bakteriosin dari beberapa galur bakteri asam laktat isolate lokal genus *Streptococcus* dan *Weissella* *Jurnal Ilmu Kefarmasian Indonesia* **9**(2) pp. 116-21.
- [6] Nurul H, Iman R, Risa R M 2016 *Karakterisasi Bakteriosin Bakteri Asam Laktat Dari Sawi Fermentasi* Thesis (Sekolah Pascasarjana, Institut Pertanian Bogor).
- [7] Yang E, Fan L, Jiang Y, Doucette C, Fillmore S 2012 Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts *AMB Express* **2**(1) pp. 48 - 59.
- [8] Yurong G, Shiru J, Qiang G, Zhilei T 2010 A novel bacteriocin with broad inhibitory spectrum produced by *Lactobacillus sake* C2, isolated from traditional Chinese fermented cabbage. *Food Control* **21**(1) pp 76-81.