Production of Crude Bacteriocin and Bacteriocin Activity from Fermented Chicken Egg

Azmi Mangalisu[1](✉), Irma Isnafia Arief[2], Andi Kurnia Armayanti[1], and Zakiah Wulandari[2]

[1] Departement of Animal Science, Faculty of Agricultural, Universitas Muhammadiyah Sinjai, Sinjai Regency, Indonesia
azmimangalisu@gmail.com

[2] Department of Animal Production Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia

Abstract. Produce bacteriocin which also has antibacterial properties. Different incubation times affect the amount of *L. plantarum* that can be identified as containing bacteriocins. Chicken eggs were pasteurized at 60 °C for 3.5 min and cracked then sterilized for 15 min and fermented with an incubation temperature of 37 °C and incubation time of 0 h, 48 h and 96 h. The parameters measured in this study were crude bacteriocin production by looking at the pH value before and after crude bacteriocin production and testing the activity of fermented chicken egg bacteriocin. Analysis of research data is analysis of variance Completely Randomized Design (CRD). The results showed that the increase in incubation time had a significant effect ($P < 0.05$) on the pH value of fermented eggs. The pH value decreased with increasing incubation time. The pH value becomes standard, namely 6.4 - 6.8 as a condition to produce crude bacteriocin. The bacteriocin activity test was carried out on *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* test bacteria. The data obtained showed that *E. coli* was sensitive and had a larger zone of inhibition when compared to *Staphylococcus aureus* and *Salmonella typhi*. The bacteriocin activity of fermented chicken eggs was optimum at the optimum incubation temperature of 37 °C for 96 h as seen from the formation of a clear zone on MHA media. The production of bacteriocin in fermented chicken eggs.

Keywords: Chicken Egg · Incubation · Bacteriocin · Fermented

1 Introduction

Chicken egg are products that contain complete and easily digestible nutrients. Chicken egg have a balanced amino acid, essential fats, several minerals and vitamins, but chicken egg are spoiled food because they are easily broken and contaminated with microbes. One way to increase the shelf life of eggs is found through the process of fermentation technology.

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Fermentation technology is used to get benefits such as functional food that is good for health, aids digestion, and extends the shelf life of a product. Fermentation technology in foodstuffs utilizing microbes has been widely used, with *Lactobacillus* bacteria being one of the most common. The research yielded promising results in the field of fermentation technology. *Lactobacillus plantarum* is a *Lactobacillus* bacteria that is often used in egg fermentation. These bacteria can grow because they need the nutrients in the growth media to survive [1].

Fermented chicken egg need to be researched on food safety. Food safety is an important thing in health insurance for consumers. Food products must be free from pathogenic microorganisms. Pathogenic microorganisms that are often found in food products include *Escherichia coli, Salmonella typhi*. Dan *Staphylococcus aureus*. These pathogenic bacteria pose a risk to the consumer's immune system so it is necessary to identify the food product to be consumed. Identification of food products that are safe for consumption, especially in fermented products, is to identify bacteriocins.

Bacteriocins are antimicrobial compounds produced from lactic acid bacteria that have the potential to be used as natural preservatives. Bacteriocins are proteins or peptides in bacteria that show bactericidal or bacteriostatic action against species that are generally closely related but there are also several types of bacteriocins that can show a wider spectrum [2]. The antagonistic properties of bacteriocins have been widely used in the field of food biopreservatives, because they have the ability to inhibit Gram positive or Gram negative bacteria. Many bacteriocins can be bactericidal against species and strains that are closely related to them, but some bacteriocins can be effective against many bacteria of different species and genera [3].

Currently, bacteriocin has begun to be applied as a biopreservative because it is natural and does not cause negative effects on consumers. Bacteriocin protein molecules are degraded by proteolytic enzymes in human digestion so they are not harmful. Bacteriocins have been used in developed countries as biopreservatives in food because they have the ability to inhibit damaging bacteria and pathogens, and do not leave residues that cause negative effects on humans [4]. Bacteriocins can inhibit the growth of pathogenic bacteria in food products so that they can be used as indicators of food safety. Based on the above study, a research was conducted on crude bacteriocin production and activity in fermented chicken eggs.

2 Materials and Methods

2.1 Materials

The used equipment in this study were sample tubes, erlenmeyer, micropipette, tip, syringe, analytical balance, measuring cup, incubator, spatula, autoclave, magnetic stirrer, vortex, lamina air flow, hot plate, centrifuge, pH meter. The used materials were mass chicken eggs, *Lactobacillus plantarum* bacterial culture, MRS (Man Rogosa Sharpe) broth, aluminum foil, tomato juice, distilled water, alcohol, CM sukrose, filter asetat selulose, media MHA, on *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli*.

2.2 Experimental Design

This research was conducted experimentally using a completely randomized design (CRD) with 3 treatments with 3 replications each. The treatments were fermented with an optimum incubation temperature of 37^0C with different incubation times of 0, 48 and 96 h.

Culture Propagation. *De Man Ragosa Sharpe* (MRS) agar was used to store *Lactobacillus. plantarum.* Subcultures are used to spread culture. Sub-culture was made by transferring the *culture stock* into liquid medium *MRS broth* (OXOID CM0359) to which 20% tomato extract was added and incubated for 24 h [5]. Cultures that had been stored in *MRS broth* media are inoculated as much as 10% into egg whites containing 20% tomato extract to produce working cultures [6].

Sample Preparation. Chicken egg samples were cleaned by using clean water. The chicken eggs were fumigated by using Calcium Permanganate (CP) powder and formalin in a closed room for 5 min and successively cleaned by using a wet cloth, chlorine solution and wiped with alcohol by using a cotton swab. Eggs were wrapped in aluminum foil and pasteurized at 60^0C for 3.5 min [7] then separated from the shell and then put into a sample bottle. The sample bottles were first cleaned by using warm water and sterilized. The 100 ml sample was homogenized before being sterilized with ultraviolet light in a *PCR* Hood for 15 min. The sterile sample was mixed with 10 ml of working culture before being homogenized with a tube shaker and fermented according to the research protocol [8].

Production of Coarse Bacteriocins from Fermented Egg Media. The isolates that had been selected and showed antimicrobial activity were grown on CM sucrose at 35°C for 48 h. The cell cultures formed were centrifuged (8,000 rpm, 4°C for 15 min) to obtain the supernatant. The cell-free supernatant was pH adjusted (pH 6.5 – 6.8) by adding 1N NaOH (with a view to removing the influence of organic acids), then filtered with 0.2 m cellulose acetate filter so that the bacteria were separated. The cell-free supernatant is a crude bacteriocin.

Bacteriocin Activity Test. The bacteriocin activity test was carried out using the agar well diffusion method. A total of 1000 µl of indicator bacteria were inoculated on MHA media, then 6 mm diameter wells were made on the solidified agar. In each well, 10 l of crude bacteriocin was added. The MHA plate was then incubated at 35^0C for 24 h. Bacteriocin activity was indicated by the presence of a clear clear/halo zone around the well. Bacteriocin activity was expressed as Arbitrary Units (AU) per ml. One AU is expressed as the area of the inhibition zone per unit volume of the tested bacteriocin sample (mm^2/ml) [9].

$$BacteriocinActivity\left(mm^2/ml\right) = \frac{Lz - Ls}{V} \tag{1}$$

Information :
Lz: clear zone area (mm^2).
Ls: area of well (mm^2).
V: sample volume (mL).

2.3 Statistical Analysis

The data from the research were tested for normality to find out the residual value was normally distributed if the significance value ($P > 0.05$) then the residual value was normally distributed. Furthermore, homogeneity test was conducted to find out that two or more groups of sample data came from populations that had the same variance, if the significance value ($P > 0.05$) then the data distribution was homogeneous. Furthermore, the data is processed used to analysis of variance (ANOVA). If the results of the analysis of treatment variance revealed a significant effect ($P < 0.05$), the LSD multiple comparison test was used. The data for testing was processed using the SPSS application.

3 Results and Discussion

The pH values of before and after neutralization of crude bacteriocins in fermented chicken eggs with different incubation times can be seen in Table 1.

Table 1 shows the initial pH conditions of the cell-free supernatant and the pH conditions of the cell-free supernatant which had been neutralized using 1N NaOH. Based on the pH values in Table 1. The antimicrobial supernatant that was produced from the production medium with the inducer was in an acidic condition. The acidic condition is caused by the presence of organic acids formed as primary metabolites of lactic acid bacteria. These organic acids have a broad spectrum of inhibition against microorganisms, namely by attacking cell walls, cell membranes, enzyme metabolism, protein synthesis systems and genetically [10].

The pH of the centrifuged cell-free supernatant was in the acidic pH range of 3.55 – 3.84. This acidic pH condition is caused by the presence of organic acids formed as a result of the main fermentation of *L. plantarum* bacteria. [11] explained that the lower the pH of the cell-free supernatant, the more organic acids contained therein. [10] added that these organic acids have a broad spectrum of inhibition against other microorganisms by attacking cell membranes, cell walls, protein synthesis systems, enzyme metabolism and genetics.

Organic acids contained in the antimicrobial supernatant of *L. plantarum* can cover the activity of bacteriocins formed when they will inhibit the indicator bacteria in the antagonistic test. Organic acids were removed by adding buffer (NaOH 1N) so that the antimicrobial supernatant reached a pH of 5.8 – 6.2. This aims to reduce the effect of organic acids contained in the antimicrobial supernatant so that it is expected to optimize the work of the bacteriocins formed.

Table 1. The pH values of before and after neutralization of crude bacteriocins in fermented chicken eggs with different incubation times

Incubation Time (hours)	The pH values of before	The pH values of after neutralization
0	3,84 ± 0,06	6,18 ± 0,06
48	3,73 ± 0,02	6,15 ± 0,09
96	3,55 ± 0,01	6,00 ± 0,03

Table 2. Bacteriocin activity in fermented chicken eggs with different incubation times

Indicator Bacteria	Time Incubation (hours)		
	0	48	96
	----------mm----------		
E.coli	3,22 ± 0,01a	5,75 ± 0,01b	6,04 ± 0,02c
Salmonella typhi	2,98 ± 0,01a	4,54 ± 0,02b	5,28 ± 0,04c
S. aureus	2,53 ± 0,01a	3,47 ± 0,02b	3,97 ± 0,05c

Note: Different superscripts in the same line showed significant differences ($P < 0.05$).

The addition of 1N NaOH was carried out to reduce the influence of organic acids contained in the supernatant, so that it can be ascertained that the inhibitory activity produced was derived from antimicrobial compounds possessed by the neutral supernatant. The results of the addition of NaOH in the cell-free supernatant showed that the pH value increased to 6.65. [12] added that the optimal pH for inhibitory activity by bacteriocins ranged from 5.8 to 6.2 where bacteriocins were able to inhibit pathogenic bacteria by 90–100%.

Fermented egg bacteriocin stored at different incubation times can inhibit the growth of indicator bacteria *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli.* This is indicated by the presence of a clear zone around the blank disc. The results of the study on bacteriocin activity in fermented chicken eggs with different incubation times are presented in Table 2.

Analysis of variance showed that incubation time had a significant effect ($P < 0.05$) on bacteriocin activity against pathogenic bacteria (*Staphylococcus aureus, Salmonella typhi,* and *E. coli*). The results of the Least Significance Different (LSD) further test showed that antimicrobial activity was significantly different ($P < 0.05$) increased in each treatment during incubation. The bacteriocin activity of fermented chicken eggs was optimum at the optimum incubation temperature of 37^0C for 96 h as seen from the formation of a clear zone on the MHA media. [13] explained that storage at 37^0C causes bacteriocin to lose its activity, related to the influence of protease enzymes that can be found in bacteriocin solutions.

The results obtained were based on the antagonistic test, namely that there was a diameter of the inhibition zone around the well. The diameter of the inhibition zone formed is the diameter of the pseudo zone. The diameter of the inhibition zone can be the diameter of the clear zone around the well which shows bactericidal properties (kills bacteria) or the diameter of the pseudo zone which is bacteriostatic (inhibits microbial growth) [2].

The bacteriocin activity test was carried out on *Staphylococcus aureus, Sal-monella typhi* and *Escherichia coli* test bacteria. The data obtained showed that *E. coli* was sensitive and had a larger zone of inhibition when compared to *Staphylococcus aureus* and *Salmonella typhi.* Based on the zone of inhibition test, the inhibition was greater for Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) when compared to Gram positive bacteria (*Staphylococcus aureus*). This indicates that the test bacteria from the

Gram negative group were more sensitive to the activity of antimicrobial compounds from lactic acid bacteria than the test bacteria from the Gram positive group. Gram-negative cell walls are thinner so that antimicrobial compounds from lactic acid bacteria will more easily enter the cell membrane, thereby damaging the cell walls of lactic acid bacteria. Gram positive bacteria have thicker cell walls so that antimicrobials will be more difficult to penetrate the cell walls of lactic acid bacteria [14].

4 Conclusions

Production of crude bacteriocin with the addition of NaOH to reduce the influence of organic acids contained in the antimicrobial supernatant so as to optimize the work of the bacteriocin formed. The bacteriocin activity of fermented chicken eggs was optimum at the optimum incubation temperature of 37^0C for 96 h as seen from the formation of a clear zone on the MHA media.

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