

Inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 toward *L. monocytogenes*, *S. thypimurium* and *E. coli*

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Abstract. Bacteriocin is a protein compound which has bactericidal ability against pathogen bacteria. This research aims to study the inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 against *Listeria monocytogenes*, *Salmonella thypimuruim* and *Escherchia coli*. The bacteriocin produce from *Lactobacillus* SCG 1223 in the MRS broth media The experimental design used was Completely Randomized Design . The variations used in this design were percentage of inoculum (5% ,10%), medium pH (4 , 6), incubation temperature (27°C , 40°C), and incubation time (4, 10 ,14 hours). Result showed that bacteriocin from *Lactobacillus* SCG 1223 had wide spectrum toward *L. monocytogenes* ,*S. thypimuruim* and *E. coli* .The highest bacteriocin activity toward *L. monocytogenes* produced by *Lactobacillus* SCG 1223 with 10% inoculum in media with initial pH 6, incubation temperature 27°C for 14 hour, toward *S. thypimurium* produced by *Lactobacillus* SCG 1223 with in media with initial pH 6, incubation temperature 40°C for 14 hour, and toward *E. coli* was 1085.81 AU/ml, produced by *Lactobacillus* SCG 1223 in MRS broth with initial pH 4, incubation temperature 40°C for 14 hour. This study is expected to find a new food preservative that can inhibit the growth of pathogenic bacteria and extend the shelf life of food. From the economic prospective of view, bacteriocin is very promising natural alternative biopreservatives.

Keywords: Bacteriocin, *Lactobacillus* SCG1223,pathogenic bacteria.

1. Introduction

One of the problems that cause food to have a short shelf life and unsecured is the contamination of spoilage and pathogens bacteria during processing, transportation and storage. To overcome this is done through the use of preservatives. In recent years consumers are more interested in natural biopreservatives. Therefore, the orientation needs to be developed natural preservatives derived from plants or produced by microorganisms.

Protection of food from spoilage and pathogenic microorganisms by lactic acid bacteria (LAB) is through producing organic acids, hydrogen peroxide, diacetyl [1] antifungal compounds such as fatty acids [2] or phenullactic acid [3] and/or bacteriocins [4]. Bacteriocins constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides [4]. The suitability is



due to the fact that this bacteriocin has been secured in food products, non-toxic in eukaryotic cells, becomes inactive by protease enzymes in the digestive tract so as to have no effect on microflora in the gastrointestinal tract and has a suitable characteristic in food processing [5]. The application of bacteriocin in food products can provide several advantages: extending shelf life, improving microbiological quality, reducing the intensity of physical treatment and the use of chemical preservatives [6,7].

Bacteriocin produced by lactic acid bacteria can be degraded by proteolytic enzymes in human digestion and not harmful to human health. In addition, bacteriocin also has a stability to the influence of pH and temperature [8,9,10,6] Bacteriocin has stable activity in acidic and alkaline conditions, so it is potentially exploited by industries that in the process involve both acidic and alkaline conditions. Bacteriocin has stable activity, even after treatment at temperature 20°C to 100°C so it is very good if used in industries that involve hot or cold conditions in the production process [11].

Survival of microbial during these processes strongly depends on the ability of the cells to adapt and become more tolerant to the environmental conditions [12]. The temperature and pH conditions that prevail during sourdough fermentations correspond to the range of conditions for good growth, acidification and bacteriocin production by *L. amylovorus* DCE 471 [1]. The pH factor of the media will affect the growth of bacterial cells will further affect the production of bacteriocin. The adsorptions of bacteriocin (nisin) by the producer cells are dependent on the pH of the culture broth [13]. The production of bacteriocin will increase with increasing pH to optimum pH and then decrease. Temperature has two opposing influences that increase the production of bacteriocin but also can kill bacterial lactic acid bacteria-producing bacteria. Additionally, the stability, solubility, and biological activity of nisin from *Lactobacillus* are dependent on the pH of the solution. In fermentation process, at pH < 6.0, more than 80% of nisin produced is released into the medium, at pH > 6.0, most of the nisin is associated with the cellular membrane, but not the cytoplasm. Solubility and stability increase drastically with the lowering of pH, in addition, nisin is stable at pH 2.0 and, at this pH, can be autoclaved at 121°C for 15 min without inactivation [14], on the other hand, in neutral and alkaline conditions nisin is almost insoluble. Therefore, the aim of this research was to study the inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 against *Listeria monocytogenes*, *Salmonella thypimuruim* and *Escherchia coli*.

2. Materials and Methods

The bacterial strains used in this study were *Lactobacillus* SCG1223 for bacteriocin production and *L. monocytogenes*, *S. thyphimurium* and *E. coli*, for assay of bacteriocin activity. Those strains were obtained from Indonesian Agency for Agricultural Research and Development.

2.1. Preparation of inoculum

Cultures are maintained at -40 °C as frozen stock cultures in equal volumes of 10% non-fat dry milk and 20% glycerol. For activation, *Lactobacillus* SCG1223 added to *de Man–Rogasa–Sharpe* (MRS) broth and incubated at 37°C for 24 hours. Bacterial activation is also performed on the *L. monocytogenes*, *S. thyphimurium* and *E. coli*.

2.2. Research Design

The experimental design used was Completely Randomized Design with four factors: percentage of inoculum, pH of medium, incubation temperature and incubation time. The variations used in this design were percentage of inoculum (5% and 10%), medium pH (4 and 6), incubation temperature (27°C and 40°C), and incubation time (4 hours, 10 hours and 14 hours). Sample code list in Table 1.

Table 1. List of sample code

Code	Percentage of inoculum (5%)			Code	Percentage of inoculum (10%)		
	pH of media	Incubation temperature (°C)	Incubation time (hour)		pH of media	Incubation temperature (°C)	Incubation time (hour)
A1	4	27	4	B1	4	27	4
A2	6	27	4	B2	6	27	4
A3	4	40	4	B3	4	40	4
A4	6	40	4	B4	6	40	4
A5	4	27	10	B5	4	27	10
A6	6	27	10	B6	6	27	10
A7	4	40	10	B7	4	40	10
A8	6	40	10	B8	6	40	10
A9	4	27	14	B9	4	27	14
A10	6	27	14	B10	6	27	14
A11	4	40	14	B11	4	40	14
A12	6	40	14	B12	6	40	14

2.3 Bacteriocin production

Fresh culture of *Lactobacillus* SCG1223 was inoculated at a rate of 5 and 10 % in MRS broth at pH 4 and 6, then incubate in shaker incubator at 27°C and 40°C for 4, 10 and 14 hours (according to Table 1). Subsequently culture was centrifuged at 10.000 rpm, 4°C for 15 minutes to produce a supernatant which was further filtered using a 0.22 µm milipore to produce a cell-free supernatant [15,16,10]. The bacteriocin activity test was performed on natural pH supernatant and neutral pH supernatant.

2.4. Bacteriocin activity test

The *Lactobacillus* SCG1223 bacteriocin activity test was performed by well agar method [17,18]. The assay plate had of Muller Hinton agar and was seeded with about 10⁶ indicator bacteria (*L. monocytogenes*, *S. typhimurium* and *E. coli*). Then 6 mm wells were bored out and each was filled with 50µl of the bacteriocin fractions. The agar plates were incubated at 37°C for 24 hours. The bacteriocin inhibitory activity against the indicator bacteria will be seen with the appearance of clear zones around the well. The bacteriocin activity unit is defined as the AU (Arbitrary unit), 1 AU representing the area of inhibition per sample volume of bacteriocin samples tested (mm²/mL).

3. Results and Discussion

The bacteriocin activity toward *L. monocytogenes* pathogenic bacteria can lead to listeriosis and are very detrimental to human health. These bacteria can grow in cold conditions so it needs serious handling for the food industry that requires cold storage. Bacteriocin in natural supernatant pH produced from *Lactobacillus* SCG1223 with 5% inoculum had the highest activity at medium pH 4, incubation temperature 27°C and incubation time 14 hours (**Figure 1**).

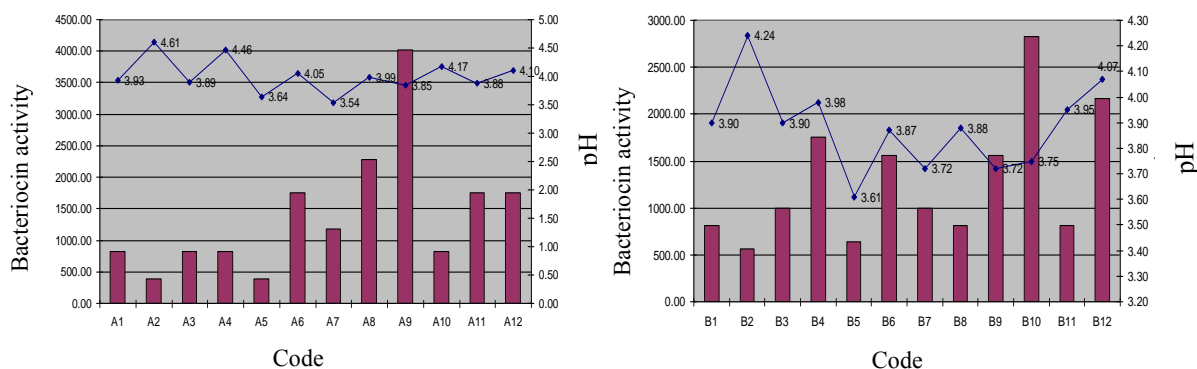


Figure1. Activity of bacteriocin in natural pH with 5% (Code A) dan 10 % (Code B) inoculum toward *L. monocytogenes*

Bacteriocin produced with 10% inoculum *Lactobacillus* SCG1223 had higher activity compared with 5% inoculum percentage (**Figure 1**). This happens because as more cells are present in the media during growth, it will further increase the production of primary metabolites. Bacteriocin is an anti-bacterial protein produced in the ribosomal pathway that occurs during the exponential phase and follows the pattern of synthesis of primary metabolites. The more cells that grow, the use of nutrients in the media will be faster, so it is suspected that with limited nutritional conditions lactic acid bacteria strain SCG 1223 get the right conditions for post-translational process that will convert prebacteriocin into active bacteriocin. Under these conditions, the highest bacteriocin activity was produced from production with a medium with an initial pH of 6, incubating at 27 °C for 14 hours.

From **Figure 1** it is seen that, bacteriocin is detected early in the exponential phase up to the stationary phase. The best results are obtained at 14 hours incubation time where the growth phase has entered the stationary phase. This shows that *Lactobacillus* SCG 1223 produces the best active bacteriocin in the stationary phase where in this phase the rate of bacterial growth is equal to the rate of death. In the stationary phase of lactic acid production as the main product of lactic acid bacteria cell metabolism begins to decline with the decrease of growth rate. In these conditions it is suspected of post-translation process in lactic acid bacteria cells. This post-translational process will affect the activity of bacteriocin produced.

3.1. The bacteriocin activity toward *S. thypimurium*

S. typhimurium is a pathogenic bacteria that can cause salmonellosis. Bacteriocin in natural supernatant pH produced from *Lactobacillus* SCG1223 with 5% inoculum had the highest activity toward *S. Typhimurium* at medium pH 6, incubation temperature 27°C and incubation time 14 hours (**Figure 2**).

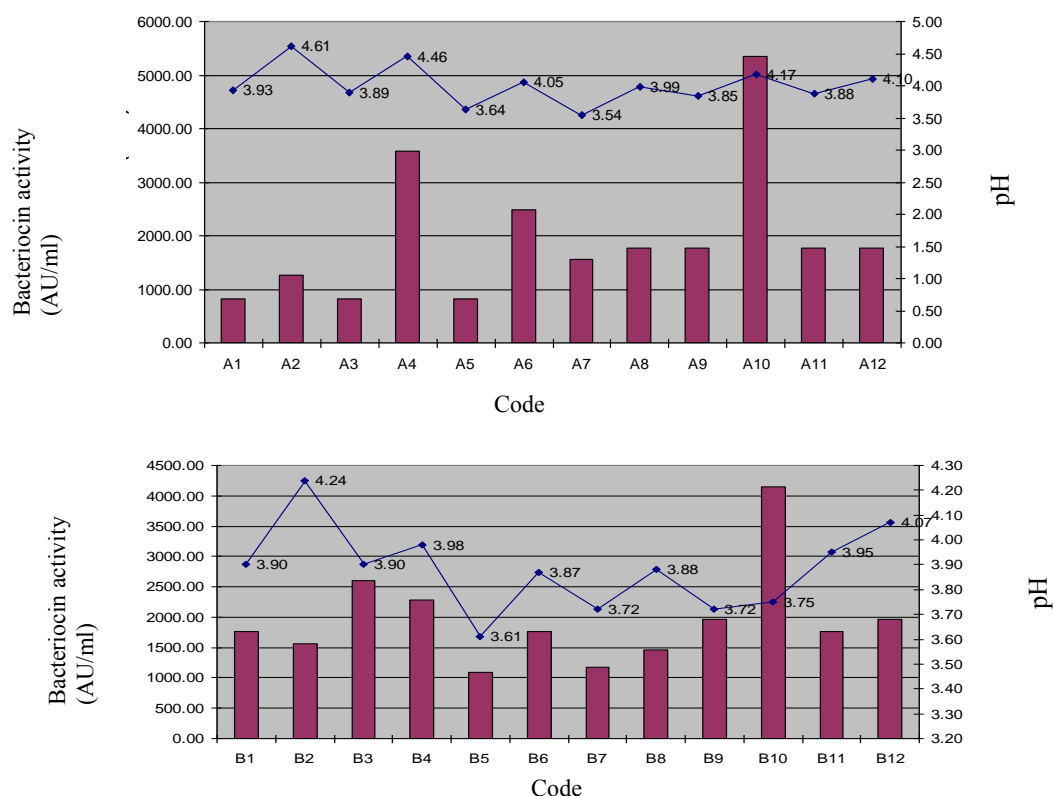


Figure2. Activity of bacteriocin in natural pH with 5% (Code A) dan 10 % (Code B) inoculum toward *S. typhimurium*

Bacteriocin produced with 10% inoculum *Lactobacillus* SCG1223 had higher activity toward *S. typhimurium* compared with 5% inoculum (Figure 5). Under these conditions, the highest bacteriocin activity was produced from production with a medium with an initial pH of 6, incubating at 27 ° C for 14 hours. These results illustrate that there is antibacterial production of compounds starting from the initial phase exponentially to the stationary phase, with the highest activity in the stationary phase. In recent years many studies have shown bacteriocin production during the stationary growth phase[19].

Lactic acid is a primary metabolite product produced in large quantities in the growth of lactic acid bacteria. Lactic acid has the ability to inhibit the growth of pathogenic bacteria. The application of lactic acid as a food preservative has been used for a long time. However, if used as a preservative in foods, lactic acid gives a sour taste and changes the texture. Bacteriocin is a natural anti-bacterial protein that also has inhibitory activity against pathogenic bacteria. Bacteriocin does not change the aroma of food and it is easily degraded by protease enzymes contained in the human body, so that its use is safe for the human body.

3.2. The bacteriocin activity toward *E. coli*

S. typhimurium is a pathogenic bacteria that can cause salmonellosis. Bacteriocin in natural supernatant pH produced from *Lactobacillus* SCG1223 with 5% inoculum had the highest activity toward *S. Typhimurium* at medium pH 6, incubation temperature 27°C and incubation time 14 hours (Figure 4).

Bacteriocin in supernatant with non-neutralized pH (pH <7) produced from *Lactobacillus* SCG1223 with all incubation treatment, provides inhibitory activity against *E.coli*. In the initial 5% inoculum use, bacteriocin produced from different treatment variations had different

activities. This high activity difference is related to the resulting metabolite product which has antagonistic properties against the indicator bacteria. Bacteriocin in natural supernatant pH produced from *Lactobacillus* SCG1223 with 5% inoculum had the highest activity toward *S. Thypimurium* at medium pH 6, incubation temperature 40°C and incubation time 14 hours (Figure 7).

When compared, at the same initial pH of the same medium and incubation temperature (treatment A4, A8 and A12) it turns out that bacteriocin produced with an incubation time of 14 hours has higher inhibitory activity compared to 10 hours and 4 hours. *Lactobacillus* SCG 1223 produces another antibacterial compound which is a secondary metabolite product in the stationary phase where in this phase the bacterial growth activity is equal to the rate of death. In some lactic acid bacteria the secondary metabolite product especially bacteriocin is produced during the exponential phase where in this phase the production of metabolites is secondary to the growth of lactic acid bacteria [19].

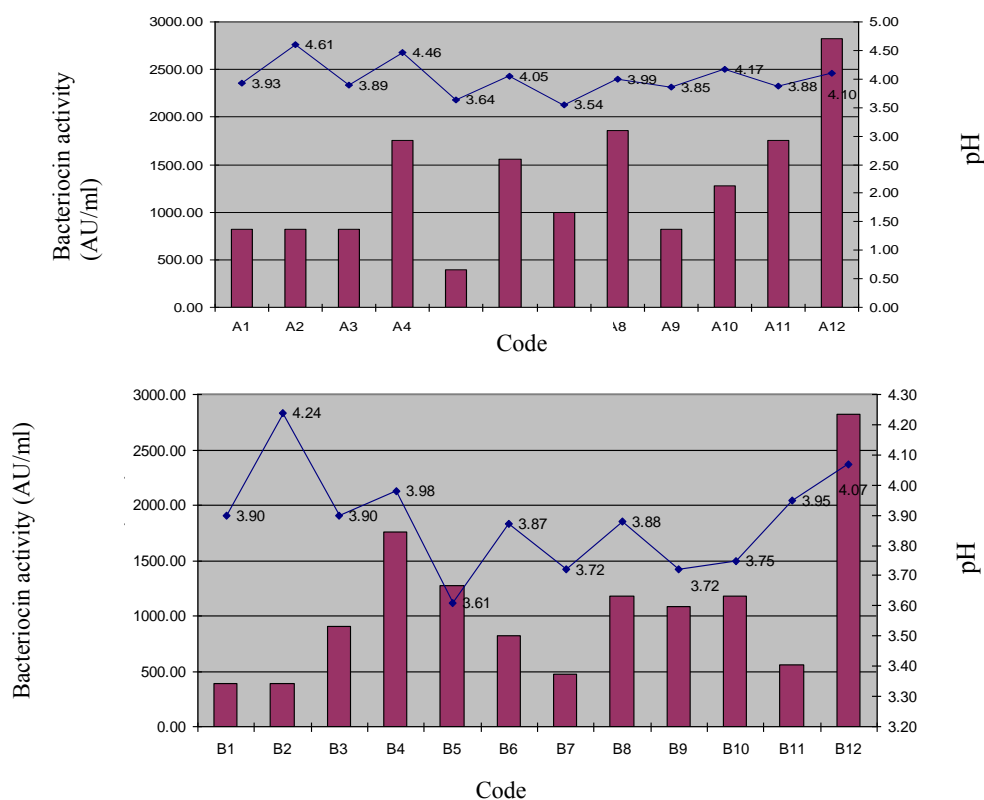


Figure 3. Activity of bacteriocin in natural pH with 5% (Code A) dan 10 % (Code B) inoculum toward *E.coli*

Bacteriocin in natural supernatant pH produced from *Lactobacillus* SCG1223 with 10% inoculum had the highest activity toward *E.coli* at medium pH 6, incubation temperature 40°C and incubation time 14 hours (Figure 3). The highest activity result is the same as in percentage of 5% inoculum which in stationary phase is produced higher activity. In the stationary phase a more active antibacterial metabolite product is produced than the metabolite product produced in the exponential phase. Based on the inhibitory test results, the higher the pH of the media and the

incubation temperature will further increase bacterial growth and also increase the activity of the antibacterial compounds produced.

Based on these results bacteriocin production which has the highest activity against *E. coli* indicator bacteria occurs during exponential phase. In the stationary phase, either at the beginning of the stationary phase and at stationary stationary phase, bacteriocin activity is detected even in lower amounts than in the stationary phase. In the stationary phase of bacteriocin which is a protein begins in production where in this phase occurs the primary metabolite process. In the stationary phase it is suspected that the bacteriocin produced during the exponential phase becomes more active with the post-translational process necessary to convert prebacteriosine into active bacteriocin.

In the exponential phase, *Lactobacillus* SCG 1223 already produces bacteriocin which is a natural protein that is antibacterial. This is indicated in the neutral supernatant inhibitory assay results, with the greatest inhibitory activity against *S. thypimurium*. The synthesis of bacteriocin by producing bacteria occurs during an exponential phase trip [20] which usually follows the pattern of synthesis of primary metabolites. This production system is governed by extracromosomal plasmids [21]. However, the bacteriocin formed does not yet have a sufficiently high and widespread activity against the three indicator bacteria. This is because the process of post-translation has not been completed where at this stage activation of prebakteriosin into active bacteriocin.

Understanding of their mechanisms and inhibitory activity on different biochemical conditions that naturally exist in food is neededn for study the effectiveness of the use of bacteriocins in food preservation [22]. In general, bacterial origin of BAL has the ability to inhibit other bacteria with bactericidal effects. Mechanism of bactericidal activity several bacteriocins in general as follows: (1) molecular bacteriocins having direct contact with the cell membrane, (2) the contact process is capable of disrupting the membrane potential in the form destabilitas cytoplasmic membrane so that the cells become strong, (3) the instability of the membrane is able to make an impact The formation of a hole or a pot in the cell membrane through a process of interference with the PMF (Proton Motive Force) [23]. The leakage that occurs due to the formation of holes in the cytoplasmic membrane is indicated by the presence of inactivity of cellular molecules. The leaks that occur have an impact on the decrease in cellular pH gradient. In general, the influence of the cytoplasm hole formation as a result of their bacteriocins, leads to changes in membrane potential gradient and the release of intracellular molecule as well as the influx of extracellular substance (environment). Its effect causes stunted cell growth and results in the process of death in cells that are sensitive to bacteriocin.

As known that lactic acid bacteria have been recognized as safe. Bacteriocins produced by LAB may be a good solution to the problem of resurgence of resistant strains to antibiotics. During the last decade, a large number of bacteriocin from LAB-have been identified, and characterized biochemically and genetically. [24]. Since most of LAB strains have a GRAS status, their addition to food should not have associated legislative problems. The strain in some cases will grow and produce bacteriocin in the food. Another advantage from an economic point of view, the cultivation of these strains for inoculation purposes is in many ways relatively inexpensive. The development of resistant populations of problematic bacteria MAYBE a potential problem associated with using bacteriocins as biopreservatives in foods [25].

4. Conclusion

From the bacteriocin activity test on the natural supernatant of *Lactobacillus* SCG 1223, there was an effect of other antibacterial compounds. The highest bacteriocin activity toward *L. monocytogenes* produced by *Lactobacillus* SCG 1223 with 10% inoculum in media with initial pH 6, incubation temperature 27°C for 14 hour, toward *S. thypimurium* produced by *Lactobacillus* SCG 1223 with in media with initial pH 6, incubation temperature 40°C for 14

hour, and toward *E. coli* was 1085.81 AU/ml, produced by *Lactobacillus* SCG 1223 in MRS broth with initial pH 4, incubation temperature 40°C for 14 hour

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