Bacteriocinogenic Lactic Acid Bacteria Isolated from Mangrove Sediment in Indonesia: Growth Optimization, Bacteriocin Production, and its Application in Food Preservation

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1. Introduction

The mangrove ecosystem is unique because it is located between marine and land ecosystems. With this condition, the mangrove ecosystem has a high diversity of microorganisms. Several studies have explored the diversity and potential of bacteria in intertidal ecosystems, for example, cellulolytic bacteria from mangrove sediment (Kurniawan et al. 2017; Ambeng et al. 2019; Nursyirwani et al. 2020; Pramono et al. 2021), mangrove vegetation (Hwanhlem et al. 2013; Hastuti et al. 2015), and surrounding ecosystems (Elayaraja et al. 2014; Maharsiwi et al. 2020).

Lactic Acid Bacteria (LAB) are known to improve the health of their host. For example, bacteriocinogenic LAB inhibits the growth of pathogenic bacteria and colonizes the human digestive tract. LAB, which can produce bacteriocins, has been reported to be successfully isolated from various samples (Meruvu and Harsa 2022), including fresh and fermented vegetables, fermented protein, dairy products, gastrointestinal tract, and soil. However, there still needs to be more research exploring lactic acid bacteria (LAB) from mangrove ecosystems.

Kusharyati et al. (2021) isolated potential LAB from mangrove sediments in Logending Beach, Central Java (Indonesia). Potential screening is carried out through producing bacteriocins and their antimicrobial activity. Bacteriocins are reported to inhibit the growth of Gram-positive and Gram-negative bacteria, both in vitro and in vivo (Heredia-Castro et al. 2015; Alajekwu and Ike 2017; Kusharyati et al. 2021). Crude extract of bacteriocins of LG71 isolate has antibacterial activity against several foodborne pathogenic bacteria, i.e., Salmonella typhi, Shigella flexneri, and Listeria monocytogenes. The LG71 isolate can be a bio-preservation agent for food, including fishery and marine products.
The potential of Lactic Acid Bacteria is species-specific, even strain-specific. Each type of bacteria has a different character, such as growth patterns, kinetics, nutritional needs, and metabolic products. The formulation of growth media is one factor influencing LAB’s growth. Adding glucose to the growth medium has been reported to increase LAB growth (Subagiyo et al. 2015). LAB uses carbohydrates as the only source of carbon for its growth (George et al. 2018). In addition, incubation time is reported to affect microbial growth and biomass production (Nurlaela et al. 2016). Yang et al. (2012) report that the formula of culture media and pH affects the LAB growth time and bacteriocin production. In this study, we aim to know the effect of glucose supplementation on the growth of LAB LG71 isolate, bacteriocin production, and its application in food bio-preservation.

2. Materials and Methods

2.1. Re-Cultivation of LAB Isolates

Lactic Acid Bacteria LG71 isolate (origin of mangrove sediment) is a collection of the Microbiology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman (details in Kusharyati et al. 2021). A total of one Ose isolate of LG71 was inoculated into 10 ml of medium de Man Rogosa and Sharpe Broth (MRSB, Oxoid). The bacterial growing medium was incubated for 48 hours at 37°C. Then, pure-activated isolates were grown on a slant MRSA (Oxoid) medium.

2.2. Characterization of LG71 Isolate

LG71 isolate was grown on an MRSA medium (48 hours at 37°C) to obtain a single colony. A single colony is characterized by observing the colony's shape, color, colony edge, elevation, and optical characteristics. Colonies confirmed as LAB colonies are carried out with Gram staining, catalase, oxidase, indole production, methyl red, Voges-Proskauer test, and oxidative-fermentative test (Cappuccino and Sherman 2014).

2.3. Production of Cell Biomass

1 ml of LG71 isolate culture (aged 48 hours) was grown into 20 ml MRSB medium and incubated for 24 hours at 37°C. A total of 2 ml of the culture is then inoculated into 4 types of production medium (medium volume 200 ml), i.e., MRSB + Glucose 0%; MRSB + Glucose 0.25%; MRSB + Glucose 0.5%; and MRSB + Glucose 1%. The production medium is incubated at different lengths of time, namely 15 hours, 18 hours, 21 hours, and 24 hours. Each treatment was repeated 3 times.

2.4. Calculation of Total Cells

The total number of cells is calculated using the Total Plate Count (TPC) method. A total of 1 ml of liquid culture of LG71 isolate from the cell biomass production medium is diluted to 10-8. A total of 1 ml of suspension from the last three dilution tubes was inoculated into MRSA medium and then incubated for 48 hours at 37°C. Growing colonies are counted. The number of cells is expressed in colony-forming units per ml (CFU/ml) as follows:

$$\text{CFU s/ml} = J K \times \frac{1}{F \text{P}}$$

Where:
- CFU s/ml = colony-forming units in 1 ml sample suspension
- JK = number of colonies
- FP = dilution factor

2.5. Measurement of Optical Density (O.D.)

Measurement of culture optical density is carried out by spectrophotometric method. 3 ml of liquid culture of LG71 isolate from the cell biomass production medium was centrifuged at 10,000 rpm for 5 minutes. Pellets are suspended with 3 ml of phosphate buffer (pH 5.3). Suspension measured its absorbance at wavelength $\lambda$=600 nm. The blanks used are phosphate buffers.

2.6. Measurement of Lactic Acid Production

Titration methods measured lactic acid production at the 15th, 18th, 21st, and 24th hours. A total of 10 ml of liquid culture of LG71 isolate from the cell biomass production medium was taken, and 3 drops of phenolphthalein indicator solution (PP 1%) were added. The sample was titrated to pink using a 1N NaOH solution. The content of lactic acid is calculated according to the following formula:

$$\% \text{ Lactic acid} = \frac{V_1 \times N \times 90}{V_2 \times 1,000} \times 10$$

Where:
- $V_1$ = NaOH volume used (ml)
- $V_2$ = titrated sample (ml)
- $N$ = Normality of NaOH solution
2.7. Preparation of Bacteriocin-Producing Medium

A total of 2 ml of liquid culture of LG71 isolate was inoculated in 2 types of medium (medium volume 200 ml), i.e., MRSB with and without glucose supplementation. The production medium was incubated at a temperature of 37°C for 18 hours. The treatment is repeated 3 times.

2.8. Evaluation of the Anti-Salmonella Activity of Bacteriocin In Vitro

The antibacterial activity of crude bacteriocin extracts at various concentrations was analyzed experimentally using a Complete Randomized Design (CRD) with 1 factor, the concentration of crude bacteriocin extracts. The parameters measured were the diameter of the inhibitory zone produced by crude extracts of bacteriocins against the test pathogenic bacteria and the number of pathogenic cells in marine processed products.

A total of 10 ml of LG71 isolate culture (aged 48 hours) was centrifuged for 10 min at 10,000 rpm. Cell-free supernatants (CFS) were tested against Salmonella typhi using Kirby’s Bauer method (Hudzicki 2009). 6 mm diameter disc paper dripped with 20 μL CFS is placed on the Nutrient Agar (Merc) medium inoculated by S. typhi. Sterile MRSB is used as a negative control. The formed inhibitory zone is observed and measured by the formula (Hendrati et al. 2017):

\[ D = \frac{D_1 + D_2}{2} \]

Where:
D = total diameter of the inhibitory zone
D1 = vertical diameter of the inhibitory zone
D2 = horizontal diameter of the inhibitory zone

2.9. Bacteriocin Extraction Using Ammonium Sulfate Precipitation Method

A total of 10 ml of LG71 isolate culture (aged 18 hours) was centrifuged at 10,000 rpm for 10 min at 4°C. The obtained supernatants are separated by salting out by adding ammonium sulfate (Sharmila and Vidya 2015). A 100 ml of culture filtrate is precipitated gradually by adding ammonium sulfate. A mixture of supernatant and ammonium sulfate is homogenized. Ammonium sulfate (50%) is added slowly until saturation ends. Bacteriocins (crude extracts) precipitation occurs as ammonium sulfate is slowly added. The crude extract of bacteriocins is separated from the liquid by centrifugation at 10,000 rpm for 15 min at 4°C. The precipitate is weighed and dissolved into 0.1 M phosphate buffer (pH 5.3) with a final concentration of 2%.

2.10. Confirmation of Crude Bacteriocin Extract

Crude extracts of bacteriocins were tested against proteolytic enzymes. Bacteriocins will lose their inhibitory activity after reacting with proteolytic enzymes (Kusumarwati et al. 2014). A total of 20 μL of crude extract (C.E.) of bacteriocins was mixed into 200 μL of proteolytic enzyme solution (Proteolytic enzyme dissolved into phosphate buffer pH 7). This mixture is incubated at a temperature of 37°C for 2 hours. After that, the mixture was tested against Salmonella typhi by Kirby’s Bauer method (Hudzicki 2009). Confirmation of bacteriocins was reported in the absence of an inhibitory zone around the disc paper.

2.11. Evaluation of Anti-Salmonella Activity In Vivo

Salmonella typhi culture was inoculated on Nutrient Broth medium for 8 hours at 37°C. A total of 0.1 ml of S. typhi culture (~10⁸ CFU/ml) was cultivated into an N.A. medium. 6 mm diameter disc paper was dripped with 20 μL of crude extract of bacteriocins of various concentrations (0.0%, 0.5%, 1.0%, 1.5%, and 2.0%). The disc paper is placed on the N.A. media inoculated by S. typhi. The bacterial growing medium was incubated for 24 hours at 37°C. Each treatment is repeated 3 times. The formed inhibitory zone is observed and measured by the formula (Hendrati et al. 2017):

\[ D = \frac{D_1 + D_2}{2} \]

Where:
D = total diameter of the inhibitory zone
D1 = vertical diameter of the inhibitory zone
D2 = horizontal diameter of the inhibitory zone

2.12. Evaluation of Anti-Salmonella Activity In Vivo

Determination of the inhibitory effect of crude bacteriocin extracts on pathogenic bacteria was tested in vivo on marine fish ball products. Fish balls are dipped in a bacteriocin solution with a
concentration of 0% (sterile aqueous), 0.5%, 1.0%, 1.5%, and 2.0% for 5 minutes. Fish balls are put in a plastic bag and stored for 6 days at 4°C. Total Plate Count (TPC) measurements are carried out before treatment and on days 3 and 6 after treatment. A total of 1 gr of fish balls was crushed. The sample is diluted with stratified dilution to 10⁻⁵. The suspensions of the last two dilutions were inoculated on N.A. medium and incubated for 48 hours at room temperature.

2.13. Data Analysis

Data on the effect of crude bacteriocin extract concentrations on anti-Salmonella activity in vitro and in vivo were analyzed using variance analysis (ANOVA) at 5% and 1% error levels. The treatment with an authentic influence is continued with the Smallest Real Difference test.

3. Results

Morphological characterization suggests that the LG71 isolate is Gram-positive and streptobacilli-shaped (Figure 1). LAB are Gram-positive, non-spore-forming bacteria, coccus-shaped cells, coccobacilli or rods, catalase-negative, and are facultatively anaerobic.

In physiological tests, LG71 isolate grows on MRSB media supplemented with 3 to 6.5% NaCl. The LG71 isolate cannot grow on a 10% NaCl-supplemented MRSB medium. Therefore, it is classified as a halophilic bacterium. The isolate is well-grown in a medium with a temperature of 4 to 45°C. Biochemical characterization shows that the LG71 isolate is catalase-positive, oxidase-negative, fermentative, and indole-positive, as well as negative in the methyl red and Voges-Proskauer tests (Table 1). Based on these characterizations, the LG71 isolate is confirmed as Lactobacillus sp.

The combination of glucose supplementation (0.0%, 0.25%, 0.5%, and 0.75%) and incubation time (15, 18, 21, and 24 hours) has not markedly shown a difference in cell biomass production by LG71 isolate. This introduction study recommends that Lactobacillus LG71 is already in the stationary phase at the 15th of incubation time (data not shown). Figure 2 shows that Lactobacillus LG71 has rapid growth. Cell biomass production experiment shows that glucose supplementation and incubation time differently affected the biomass production of LG71 isolate cells (p<0.01). The results of the different bacterial populations for each treatment indicate it. The supplementation of 0.25% glucose to MRSB

Figure 1. Colony characterization of LG71 isolate on MRSA medium (left image, 24 hours-aged colonies), the single purified colony has a round shape, convex surface, white color, flat edges, and medium size. The observation of cell morphology by Gram staining (right image, 1,000x magnification on light-microscope), Gram-positive bacteria, rod-shaped cells, and streptobacilli arrangement
medium and an incubation time of 15 hours is the best treatment for the growth of LG71 isolate. Combining such treatments produces the largest cell biomass (Figure 2A). The same pattern is also seen in the optical density parameters of the culture. The 0.25% glucose supplementation and 15 hours of incubation time show the highest culture optical density (Figure 2B).

Figure 3 shows that lactic acid production tends to drop for all treatments at the addition of incubation time. Although, glucose supplementation of 1% briefly raised lactic acid production at the 12th hour of incubation time. Based on the sugar fermentation pattern, there are two types of LABs: homofermentative and heterofermentative. *Lactobacillus* LG71 is suspected to be a heterofermentative LAB. Therefore, the main product of its metabolism is not only lactic acid.

Crude extracts of bacteriocins show inhibitory activity against *Salmonella typhi*. It is indicated by forming an inhibitory zone around the disc paper (Figure 4A). Crude extracts of bacteriocins exposed to the proteolytic enzyme papain have lost their inhibitory ability. It is indicated by the absence of clear zones around the disc paper (Figure 4B). These results confirm that the crude extract obtained from ammonium sulfate precipitation is bacteriocin.

Adding protease enzymes damages bacteriocin and eliminates their activity, characterized by losing the inhibition zone against test bacteria. The results of the variance analysis showed that the concentration of crude extracts of bacteriocins affected the inhibitory activity against *Salmonella typhi*, *p*<0.01 (Table 2). The higher the concentration of crude bacteriocins, the greater the inhibitory zone formed.

In the present study, 2% crude bacteriocin extract was the best concentration inhibiting *Salmonella typhi*. Crude extracts of bacteriocins show potential for bio-preservation. It is shown by in vivo tests on fish balls. In general, the third day of incubation showed that the treatment of applying crude extracts reduced the bacterial population in fish balls. Still, on the sixth day, the bacterial population increased again. The population of *S. typhi* in fish balls treated with 1.5% and 2.0% bacteriocin crude extract was still lower than the control (without applying the crude bacteriocin extract) on day 6 (Figure 5).

The results of the variance analysis showed that the bacteriocin concentration and incubation time showed different responses to *S. typhi* populations in fish balls (*p*<0.01). The administration of a 2% crude bacteriocin extract consistently indicates the lowest bacterial population at each incubation time. Although the 6th day experienced an increase in the *S. typhi* population, it was still lower than other treatments. It shows that a crude extract of 2% bacteriocins is the best concentration to suppress the growth of *S. typhi* populations in fish balls.

### Table 1. Morphological, physiological, and biochemical characteristics of isolate LG71

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Characters</th>
<th>Result</th>
<th>Reference of genus <em>Lactobacillus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Salvetti et al. 2012; Zheng et al. 2020)</td>
</tr>
<tr>
<td><strong>Physiology</strong></td>
<td>NaCl 10%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaCl 6.5%</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaCl 5%</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaCl 3%</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature 4°C</td>
<td>+</td>
<td>Temperature range 5-53°C</td>
</tr>
<tr>
<td></td>
<td>Temperature 30°C</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature 37°C</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature 45°C</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indole</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methyl Red</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voges-Proskauer</td>
<td>-</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>Oxidase</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Catalase</td>
<td>+</td>
<td>(but rare strains decompose peroxide)</td>
</tr>
<tr>
<td></td>
<td>O/F</td>
<td>Fermentative</td>
<td>Fermentative</td>
</tr>
<tr>
<td></td>
<td>Gram</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td>Cell Shape</td>
<td>Rod</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell Arrangement</td>
<td><em>Streptobacilli</em></td>
<td>Chain formation common</td>
</tr>
</tbody>
</table>

(+): Positive, (-): Negative, ND: No data
Figure 2. (A) Total population of *Lactobacillus* LG71 with different glucose supplementation and incubation time. Glucose supplementation of 0.25% resulted in the most cell populations, and (B) the absorbance value of *Lactobacillus* LG71 culture at $\lambda$600 nm with different glucose supplementation and incubation time. Glucose supplementation of 0.25% showed the highest optical density of the culture.

Figure 3. The lactic acid production by *Lactobacillus* LG71 culture at different glucose supplementation and incubation time. Lactic acid production tends to drop for all treatments at an additional incubation time.
Table 2. Analysis of the effect of crude extracts of *Lactobacillus* LG71 bacteriocin concentrations on the inhibitory activity against *Salmonella typhi* in vitro

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Diameter of the inhibitory zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriocin extract 0%</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td>Bacteriocin extract 0.5%</td>
<td>0.82±0.03b</td>
</tr>
<tr>
<td>Bacteriocin extract 1%</td>
<td>1.13±0.06bc</td>
</tr>
<tr>
<td>Bacteriocin extract 1.5%</td>
<td>1.33±0.16c</td>
</tr>
<tr>
<td>Bacteriocin extract 2%</td>
<td>1.45±0.10d</td>
</tr>
<tr>
<td>Chloramphenicol 30 ppm</td>
<td>1.85±0.30d</td>
</tr>
</tbody>
</table>

The letter behind the diameter of the inhibition zone indicates the results of further tests.

Figure 4. (A) Anti-Salmonella activity of 20 μL crude extract of bacteriocins against *Salmonella typhi* culture (~10⁸ CFU/ml) (1. bacteriocin extract 0.5%, 2. bacteriocin extract 1%, 3. bacteriocin extract 1.5%, 4. bacteriocin extract 2%, 5. chloramphenicol 30 ppm, 6. bacteriocin extract 0%), and (B) confirmation test of crude extract of bacteriocins using protease enzyme.

Figure 5. Evaluation of the inhibitory activity of crude extracts of *Lactobacillus* LG71 bacteriocin against *S. typhi* populations on fish balls for 6 days. The initial *S. typhi* population in fish balls was 3.40±0.28 Log10 CFU/g.
4. Discussion

Seafood contains omega-3, selenium, vitamins, protein, zinc, iron, magnesium, minerals, and potassium. Seafood consumption is believed to improve human health, such as support cell regeneration (Emmet et al. 2013) and reduce the risk of cardiovascular disease (Zarraquin et al. 2014). Seafood consumption continues to increase from year to year. The Indonesian Ministry of Maritime and Fisheries Affairs recorded that the national fish consumption rate 2021 reached 55.37 kg/capita, equivalent to fresh whole fish. It increased compared to the previous year, which was 54.56 kg/capita, equivalent to fresh whole fish. By 2024, the fish consumption rate in Indonesia is targeted to reach 62.5 kg/capita, equivalent to fresh whole fish.

Food safety needs to be continuously improved amid the high fishery product consumption. Salmonella spp. is a pathogenic bacterium that commonly contaminates fishery products (Elbashir et al. 2018). Yeni et al. (2017) found the prevalence of Salmonella spp. (32%; 45/141 samples) in fresh fishery products obtained from traditional and modern markets in the DKI Jakarta and Bogor areas. The highest prevalence of Salmonella spp. was successively found in shellfish, fresh shrimp, fish, and squid products. Moreover, one isolate was resistant to erythromycin, tetracycline, amoxicillin-clavulanic acid, and nalidixic acid.

Salmonella infections from seafood are commonly associated with raw, undercooked, and inadequately prepared finfish and crustaceans (Elbashir et al. 2018). Salmonella releases enterotoxins, which induce inflammation and diarrhea. Symptoms usually appear 12 to 72 hours after consuming contaminated food. Depending on the individual host variety, ingested amount, and strains, acute symptoms may last 1-2 days or longer (WHO 2018).

Gram-negative organisms cause most foodborne illnesses worldwide (Elbashir et al. 2018). Antibiotics are often used to suppress pathogenic bacterial contamination in seafood products. However, researchers reported an increase in bacterial resistance to various antimicrobial compounds. Usually, Gram-negative bacteria are naturally resistant to an antimicrobial substances due to their outer membrane, which acts as an effective barrier (Gyawali and Ibrahim 2014; Kusharyati et al. 2021). Bacteriocins, antimicrobial compounds, began to be applied in various food products (Todorov et al. 2011; Balcunas et al. 2013; Heredia-Castro et al. 2015). Bacteriocins are considered safer compared to the long-term use of antibiotics. Bacteriocins used in conjunction with chemical or physical treatments broaden their scope of action on Gram-negative bacteria while also reducing the formation of resistant cells (Ghaly et al. 2010; Prudencio et al. 2015).

Ribosomes produce and synthesize Bacteriocins during the initial bacterial growth phase, including the Lactic Acid Bacteria (LAB) group. During fermentation, LAB does not produce only lactic acid but is also known to excrete compounds with antimicrobial activity, including bacteriocins (Jiang et al. 2012; Cizeikiene et al. 2013). LAB with the ability to produce bacteriocins has been reported to be successfully isolated from various samples, including fresh and fermented vegetables (Magnusson and Schnürer 2001; Gao et al. 2010), dairy products (Voulgari et al. 2010; Luo et al. 2011), the gastrointestinal tract of mammalian (Cui et al. 2012; Kusharyati et al. 2020), and soil (Yanagida et al. 2006; Klibi et al. 2012). However, little research is still exploring lactic acid bacteria from mangrove ecosystems.

The LG71 isolate is successfully isolated from the sediment of mangrove ecosystems on the coast of Logending, Jawa Tengah (Indonesia). The characterization results showed that the LG71 isolate is confirmed as Lactobacillus sp. An interesting result, the LG71 isolate is catalase-positive. This character is rarely found in lactic acid bacteria. Generally, Lactobacillus is catalase-negative, but some other Lactobacillus produce very small catalase (Tomusiak-Plebanek et al. 2018; Zheng et al. 2020). Rare strains of Lactobacillus can decompose peroxide by pseudo-catalase (Salvetti et al. 2012; Zheng et al. 2020). For example, Lactobacillus hilgardii A33-1 and A48-1 and Lactobacillus plantarum CECT 221 were reported to have catalase activity in MRS + 2% glucose medium (Herrero et al. 1996).

Lactobacillus LG71, native to mangrove sediment, has antibacterial activity against Gram-negative pathogenic bacteria in vitro (Kusharyati et al. 2021). Similarly, Hwanhlem et al. (2013) isolated bacteriocin-producing LAB from Southern Thailand mangrove sediments and has been tested to inhibit foodborne pathogens, both Gram-positive and negative bacteria. In this present research, we optimized the growth of
The production of bacteriocins by LAB occurs at 2018). and metabolic products (George et al. convert carbon sources in the substrate into biomass is determined by the ability of microorganisms to correlation of biomass with the number of substrates used by microorganisms for biomass growth, cell maintenance, and product formation. The substrate added to the medium is generally used by microorganisms for biomass growth, cell maintenance, and product formation. The correlation of biomass with the number of substrates is determined by the ability of microorganisms to convert carbon sources in the substrate into biomass and metabolic products (George et al. 2018).

The production of bacteriocins by LAB occurs at the end of the exponential phase. Crude extracts of bacteriocins show potential for bio-preservation. A 2% crude extract of bacteriocins is the best concentration in inhibiting Salmonella typhi in vitro (p<0.01). Research on the antimicrobial activity of bacteriocin against Salmonella has been widely reported, including the application of carboxyclic A, carno-bacteriocin BM1, and piscicolin from Carnobacterium maltaromaticum UAL307 (Martin-Visscher et al. 2011).

The evaluation of anti-Salmonella activity in vivo was performed on fish balls. In general, the third day of incubation showed that the treatment of applying crude extracts suppressed the S. typhi population in fish balls. Still, on the sixth day, the S. typhi population increased again. Application of 2% crude bacteriocin extracts consistently showed the lowest S. typhi population at each incubation time (Figure 5). Similarly, Cod fillet products treated with bacteriocins contain a lower population of bacteria than those without bacteriocin treatment at 4°C (Sarika et al. 2012). Enterocin also inhibits bacterial growth in fish products stored at 4°C (Dicks et al. 2006).

Bacteriocins are generally species-specific, even strain-specific. Therefore, exploration of LAB potential from various sources needs to perform. In addition, developing bacteriocin potential as food bio-preservation needs supporting data, such as toxicology. Genomic exploration can identify bacteriocin-encoding genes and efforts to improve their expression.

In conclusion, supplementing 0.25% glucose to MRSB medium and an incubation time of 15 hours is the best treatment for producing Lactobacillus LG71 isolate cell biomass. A 2% concentration of crude extract of Lactobacillus LG71 bacteriocins is the best concentration against Salmonella typhi both during in vitro and in vivo tests in fish balls. The addition of glucose affects the production of Lactobacillus LG71 isolate cell biomass, and the bacteriocin derived from Lactobacillus LG71 gives increased protection against S. typhi and offers an alternative for food preservation.

Conflict of Interest

The author declares that there was no conflict of interest in this study.

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