HAYATI
Journal of Biosciences

Vol. 30 No. 6, November 2023 1121-1131
DOI:10.4308/hjb.30.6.1121-1131
ISSN: 1978-3019
EISSN: 2086-4094

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

Dyah Fitri Kusharyati[1*], Oedjijono[1], Taruna Dwi Satwika[1], Dwiana Muflihah Yulianti[1], Afifah Mariana[1], Anwar Rovik[2]

[1]Department of Microbiology, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Banyumas 53122, Indonesia
[2]Center for Tropical Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Sleman, Yogyakarta 53288, Indonesia

ARTICLE INFO

Article history:
Received March 6, 2023
Received in revised form June 23, 2023
Accepted June 27, 2023

KEYWORDS:
anti-Salmonella,
bacteriocin,
food preservation,
Lactobacillus,
mangrove sediment

ABSTRACT

The mangrove ecosystem is unique because it is located between marine and land. Little research is exploring lactic acid bacteria (LAB) from mangrove ecosystems. The LAB LG71 isolate is successfully isolated from the sediment of mangrove ecosystems on the coast of Logending, Jawa Tengah (Indonesia). In this study, we aimed to know the effect of glucose supplementation on the growth of LG71 isolate, bacteriocin production, and its application in food preservation. The characterization results showed that the LG71 isolate is *Lactobacillus* sp. Interestingly, the LG71 isolate is catalase-positive since this character is rarely found in the LAB group. 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 cell biomass, and the bacteriocin derived from *Lactobacillus* LG71 gives increased protection against *S. typhi* and offers an alternative for food preservation.

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.

* Corresponding Author
E-mail Address: dfitri.k@gmail.com

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:

$$\text{CFU s/ml} = \text{JK} \times \frac{1}{\text{FP}}$$

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 λ600 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:

$$\% \text{ Lactic acid} = \frac{V1 \times N \times 90}{V2 \times 1{,}000} \times 10$$

Where:

V1 = NaOH volume used (ml)
V2 = 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):

$$D = \frac{D1 + D2}{2}$$

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 Vitro*

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 (~10^8 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):

$$D = \frac{D1 + D2}{2}$$

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

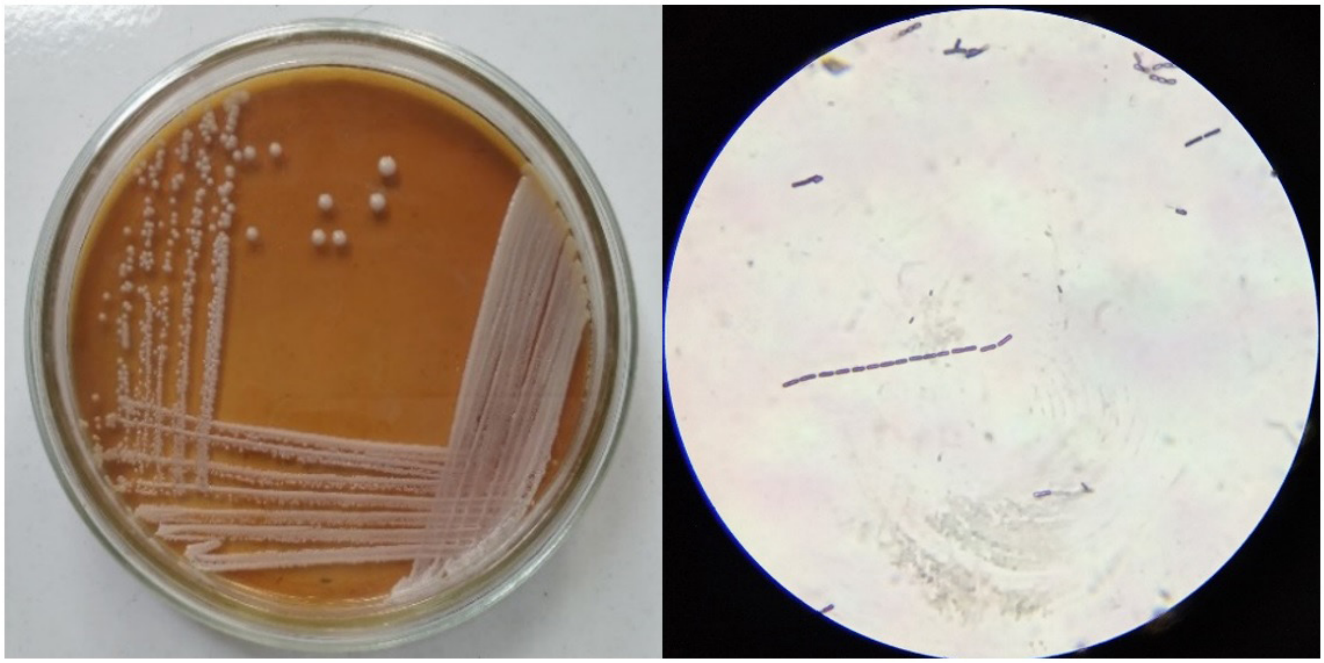


Figure 1. Colony characterization of LG71 isolate on MRSA medium (left image, 24 hours-aged colonies), the single purified colony has a round shape, convex surface, white color, flat edges, and medium size. The observation of cell morphology by Gram staining (right image, 1,000x magnification on light-microscope), Gram-positive bacteria, rod-shaped cells, and streptobacilli arrangement

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

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

(+): Positive, (-): Negative, ND: No data

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.

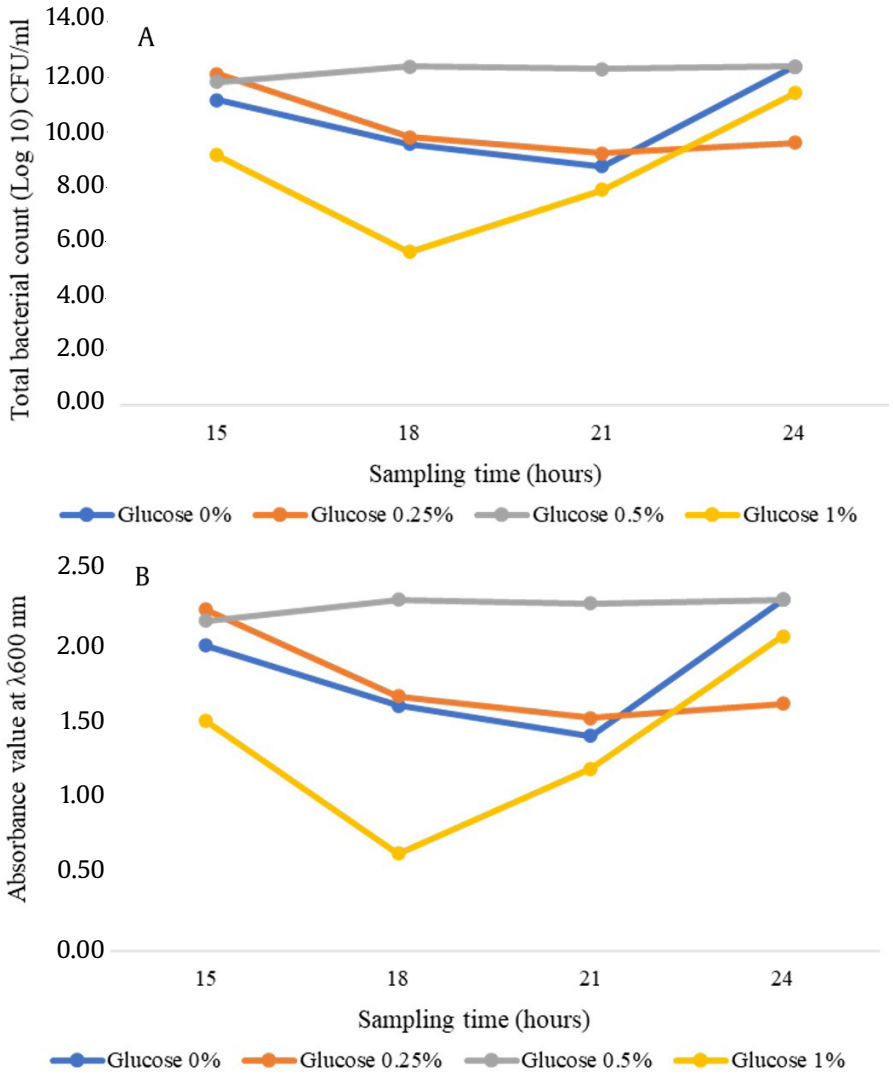


Figure 2. (A) Total population of *Lactobacillus* LG71 with different glucose supplementation and incubation time. Glucose supplementation of 0.25% resulted in the most cell populations, and (B) the absorbance value of *Lactobacillus* LG71 culture at λ600 nm with different glucose supplementation and incubation time. Glucose supplementation of 0.25% showed the highest optical density of the culture

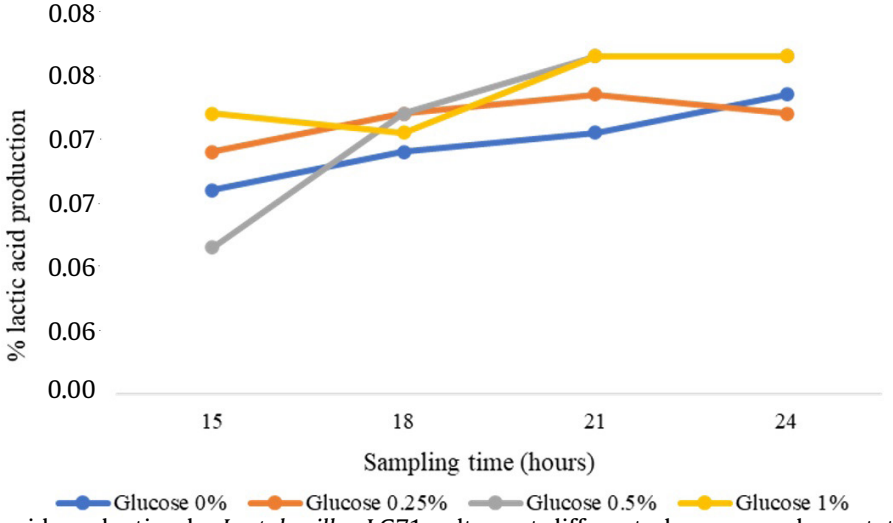
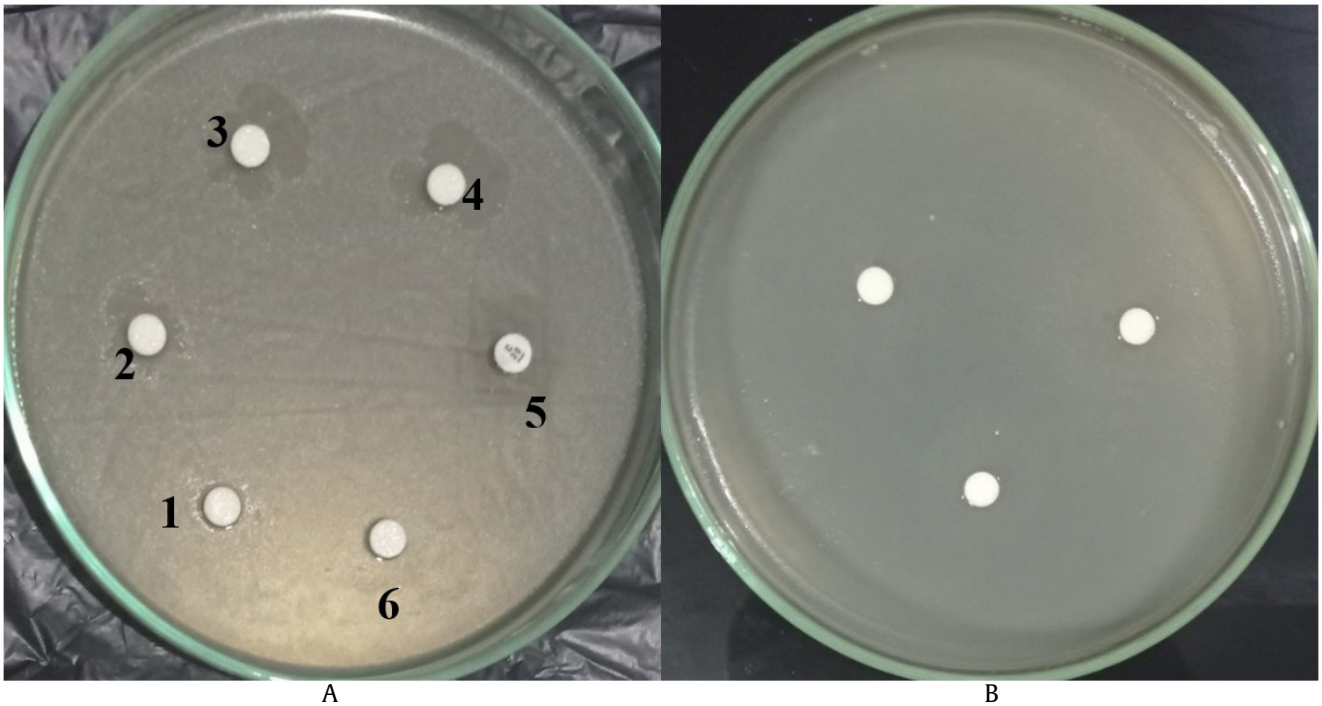


Figure 3. The lactic acid production by *Lactobacillus* LG71 culture at different glucose supplementation and incubation time. Lactic acid production tends to drop for all treatments at an additional incubation time



A B

Figure 4. (A) Anti-Salmonella activity of 20 µL crude extract of bacteriocins against *Salmonella typhi* culture (~10^8 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

Table 2. Analysis of the effect of crude extracts of *Lactobacillus* LG71 bacteriocin concentrations on the inhibitory activity against *Salmonella typhi in vitro*

Treatments	Diameter of the inhibitory zone (cm)
Bacteriocin extract 0%	0.00±0.00[a]
Bacteriocin extract 0.5%	0.82±0.03[b]
Bacteriocin extract 1%	1.13±0.06[bc]
Bacteriocin extract 1.5%	1.33±0.16[c]
Bacteriocin extract 2%	1.45±0.10[c]
Chloramphenicol 30 ppm	1.85±0.30[d]

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

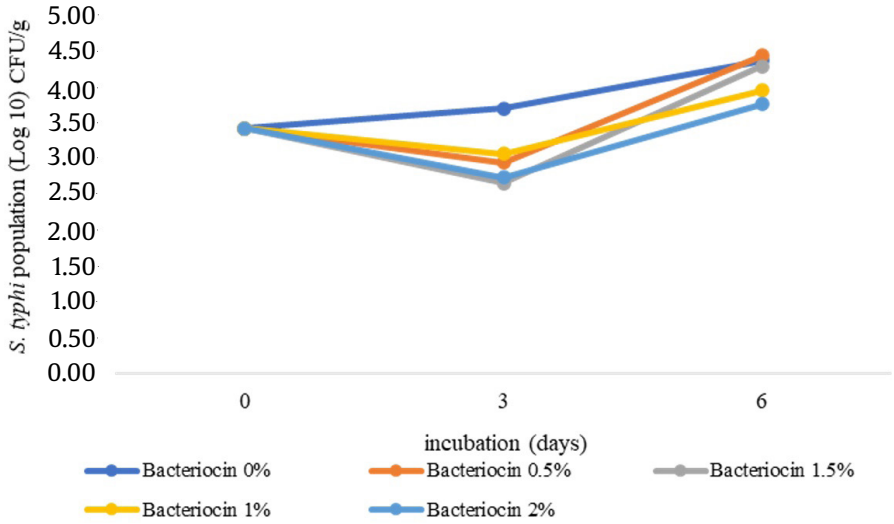


Figure 5. Evaluation of the inhibitory activity of crude extracts of *Lactobacillus* LG71 bacteriocin against *S. typhi* populations on fish balls for 6 days. The initial *S. typhi* population in fish balls was 3.40±0.28 Log10 CFU/g

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; Balciunas *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

Lactobacillus LG71 and evaluated the anti-Salmonella activity *in vivo*.

Cell biomass production experiments showed that glucose supplementation and incubation time differently affected the biomass production of *Lactobacillus* LG71 cells ($p<0.01$). This result is interesting because LAB is commonly known as slow-growing bacteria. However, the LG71 isolate is a fast-growth LAB. Our study also showed a lower need for glucose supplementation to increase the growth of LAB isolates. Subagiyo *et al.* (2015) reported that adding 1% glucose affected increasing cell biomass after 18 hours and 24 hours of growth. 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 carbocyclic 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.

Acknowledgments

The author thanks Universitas Jenderal Soedirman (Unsoed) for the funding support through the scheme of Riset Dasar Unsoed in 2022.

References

Alajekwu, O.U., Ike, A.C., 2017. Effect of bacteriocin-like inhibitory substances produced by vaginal *Lactobacilli* on group B *Streptococcus. Nig. J. Biotech.* 32, 20-27. https://doi.org/10.4314/njb.v32i1.4

Ambeng, Z.H., Oka, N.P., Tonggiroh, A., 2019. Isolation and characterization of bacteria from mangrove sediment at the coastal area in Pangkep South Sulawesi. *J. Phys. Conf. Ser.* 1341, 022016. https://doi.org/ 10.1088/1742-6596/1341/2/022016.

Balciunas, E.M., Martinez, F.A.C., Todorov, S.D., Franco, B.D.G., Converti, A., Oliveira, R.P.S., 2013. Novel biotechnological applications of bacteriocins: a review. *Food Control.* 32, 134-142. https://doi.org/10.1016/j.foodcont.2012.11.025

Cappuccino, J.G., Sherman, N., 2014. *Manual Laboratorium Mikrobiologi Edisi Kedelapan*. EGC, Jakarta.

Cizeikiene, D., Juodeikiene, G., Paskevicius, A., Bartkiene, E., 2013. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganisms isolated from food and their control in wheat bread. *Food Control*. 31, 539-545. https://doi.org/10.1016/j.foodcont.2012.12.004

Cui, Y., Zhang, C., Wang, Y., Shi, J., Zhang, L., Ding, Z., Qu, X., Cui, H., 2012. Class IIa bacteriocins: diversity and new developments. *Int. J. Mol. Sci.* 13, 16668-16707. https://doi.org/10.3390/ijms131216668

Dicks, L.M.T., Todorov, S.D., van der-Merwe, M.P., Dalton, A., Hoffman, L.C., 2006. Preservation of fish spread with enterocins 1071A and 1071B, two antimicrobial peptides produced by *Enterococcus faecalis* BFE 1071. *J. Food Safety*. 26, 173–183. https://doi.org/10.1111/j.1745-4565.2006.00041.x

Elayaraja, S., Annamalai, N., Mayavu, P., Balasubramanian, 2014. Production, purification, and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. *Asian Pac. J. Trop. Biomed.* 4, 305-311. https://doi.org/10.12980/APJTB.4.2014C537

Elbashir, S., Parveen, S., Schwarz, J., Rippen, T., Jahncke, M., DePaola, A., 2018. Seafood pathogens and information on antimicrobial resistance: a review. *Food Microbiol.* 70, 85-93. https://doi.org/10.1016/j.fm.2017.09.011

Emmett, R., Akkersdyk, S., Yeatman, H., Meyer, B.J., 2013. Expanding awareness of docosahexaenoic acid during pregnancy. *Nutrients.* 5, 1098-1109. https://doi.org/10.3390/nu5041098

Gao, Y., Jia, S., Gao, Q., Tan, Z., 2010. A novel bacteriocin with a broad inhibitory spectrum produced by *Lactobacillus sakei* C2, isolated from traditional Chinese fermented cabbage. *Food Control.* 21, 76-81. https://doi.org/10.1016/j.foodcont.2009.04.003

George, F., Daniel, C., Thomas, M., Singer, E., Guilbaud, A., Tessier, F.J., Revol-Junelles, A.M., Borges, F., Foligne, B., 2018. Occurrence and dynamism of lactic acid bacteria in distinct ecological niches: a multifaceted functional health perspective. *Front. Microbiol.* 9, 2899. https://doi.org/10.3389/fmicb.2018.02899

Ghaly, A.E., Dave, D., Budge, S., Brooks, M.S., 2010. Fish spoilage mechanism and preservation technique: review. *American J. Appl. Sci.* 7, 859-877.

Gyawali, R., Ibrahim, S.A., 2014. Natural products as antimicrobial agents. Food Control. 46, 412-429. DOI: 10.1016/j.foodcont.2014.05.047

Hastuti, S.U., Sangur, K., Khasanah, H.N., 2015. Biodiversity and enzyme activity of indigenous cellulolytic and amylolytic bacteria in decayed mangrove stem waste product at Wadai Seashore, Ambon Island. *J. Agric. Sci. Technol.* 5, 252-256. https://doi.org/10.17265/2161-6264/2015.04.004

Hendrati, P.M., Kusharyati, D.F., Ryandini, D., Oedjijono, 2017. Characterization of bifidobacteria from infant feces with different modes of birth at Purwokerto, Indonesia. *Biodiversitas.* 18, 1265-1269. https://doi.org/10.13057/biodiv/d180352

Heredia-Castro, P.Y., Mendez-Romero, J.I., Hernandez-Mendoza, A., Acedo-Felix, E., Gonzales-Cordova, A.F., Vallejo-Cordoba, B., 2015. Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances produced by *Lactobacillus* spp. isolated from artisanal Mexican cheese. *J. Diary. Sci.* 98, 8285-8293. https://doi.org/10.3168/jds.2015-10104

Herrero, M., Mayo, B., Gonzales, B., Suarez, J.E., 1996. Evaluation of technologically important traits in lactic acid bacteria isolated from spontaneous fermentations. *J. Appl. Bacteriol.* 81, 565-570.

Hudzicki, J., 2009. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. Available at: https://asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro. [Date accessed: 29 December 2022]

Hwanhlem, N., Biscola, V., El-Ghaish, S., Jaffres, E., Dousset, X., Haertle, T., Kittikun, A.H., Chobert, J., 2013. Bacteriocin-producing lactic acid bacteria isolated from mangrove forests in southern Thailand as potential bio-control agents: purification and characterization of a bacteriocin produced by *Lactococcus lactis* subsp. lactis KT2W2L. *Probiotics Antimicrob. Prot.* 5, 264-278. https://doi.org/10.1007/s12602-013-9150-2

Jiang, J., Shi, B., Zhu, D., Cai, Q., Chen, Y., Li, J., Qi, K., Zhang, M., 2012. Characterization of a novel bacteriocin produced by *Lactobacillus sakei* LSJ618 isolated from traditional Chinese fermented radish. *Food Control.* 23, 338-344. https://doi.org/10.1016/j.foodcont.2011.07.027

Klibi, N., Slimen, N.B., Fhoula, I., Lopez, M., Salma, K.B., Daffonchio, D., Boudabous, A., Torres, C., Ouzari, H., 2012. Genotypic diversity, antibiotic resistance, and bacteriocin production of enterococci isolated from rhizospheres. *Microbes Environ.* 27, 533–537. https://doi.org/10.1264/jsme2.ME12041

Kurniawan, A., Prihanto, A.A., Sari, S.P., Febriyanti, D., Kurniawan, A., Sambah, A.B., Asriani, E., 2017. Isolation and identification of cellulolytic bacteria from mangrove sediment in Bangka Island. *IOP Conf. Ser. Earth Environ. Sci.* 137, 012070. https://doi.org/10.1088/1755-1315/137/1/012070

Kusharyati, D.F., Pramono, H., Ryandini, D., Manshur, T.A., Dewi, M.A., Khatimah, K., Rovik, A., 2020. Bifidobacterium from infant stool: the diversity and potential screening. *Biodiversitas.* 21, 2506-2513. https://doi.org/10.13057/biodiv/d210623

Kusharyati, D.F., Oedjijono, Satwika, T.D., Mariana, A., Rovik, A., 2021. Potential screening of bacteriocinogenic-lactic acid bacteria from mangrove sediment of Logending Beach for fisheries product preservation. *JTBB.* 6, jtbb61927. https://doi.org/10.22146/jtbb.61927

Kusmarwati, A., Arief, F.R., Haryati, S., 2014. Eksplorasi bakteriosin dari bakteri asam laktat asal rusip Bangka dan Kalimantan. *JPB Perikanan.* 9, 29-40. https://doi.org/10.15578/jpbkp.v9i1.97

Luo, F., Feng, S., Sun, Q., Xiang, W., Zhao, J., Zhang, J., 2011. Screening for bacteriocin-producing lactic acid bacteria from kuru, a traditional naturally-fermented yak milk from Qinghai Tibet Plateau. *Food Control.* 22, 50-53. https://doi.org/10.1016/j.foodcont.2010.05.006

Magnusson, J., Schnurer, J., 2001. *Lactobacillus coryniformis* subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Appl. Environ. Microbiol.* 67, 1-5. https://doi.org/10.1128/AEM.67.1.1-5.2001

Maharsiwi, W., Astuti, R.I., Meryandini, A., Wahyudi, A.T., 2020. Screening and characterization of sponge-associated bacteria from Seribu Island, Indonesia producing cellulase and laccase enzymes. *Biodiversitas.* 21, 975-981. https://doi.org/10.13057/biodiv/d210317

Martin-Visscher, L.A., Yoganathan, S., Sit, C.S., Lohans, C.T., Vederas, J.C., 2011. The activity of bacteriocins from *Carnobacterium maltaromaticum* UAL307 against gram-negative bacteria in combination with EDTA treatment. *FEMS Microbiol. Lett.* 317, 152-159. https://doi.org/10.1111/j.1574-6968.2011.02223.x

Meruvu, H., Harsa, S.T., 2022. Redefining methods for augmenting lactic acid bacteria robustness and phenyl lactic acid biocatalysis: integration valorizes simplicity. *Critical Rev. Food Sci. Nutr.* 0, 1-13. https://doi.org/10.1080/10408398.2022.2054936

Nurlaela, S., Sunarti, T.C., Meryandini, A., 2016. Formula medium pertumbuhan bakteri asam laktat *Pediococcus pentosaceus* menggunakan substrat whey tahu. *J. Sumberdaya HAYATI.* 2, 31-38. https://doi.org/10.29244/jsdh.2.2.31-38

Nursyirwani, N., Feliatra, F., Tanjung, A., Harjuni, F., 2020. Isolation of cellulolytic bacteria from mangrove sediment in Dumai marine station Riau and the antibacterial activity against pathogens. *IOP Conf. Ser. Earth Environ. Sci.* 430, 012012. https://doi.org/10.1088/1755-1315/430/1/012012

Pramono, H., Mariana, A., Ryandini, D., Sudiana, E., 2021. Diversity of cellulolytic bacteria isolated from coastal mangrove sediment in Logending Beach, Kebumen, Indonesia. *Biodiversitas.* 22, 1869-1878. https://doi.org/10.13057/biodiv/d220433

Prudencio, C.V., dos-Santos, M.T., Vanetti, M.C.D., 2015. Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology. *J. Food Sci. Technol.* 52, 5408–5417. https://doi.org/10.1007/s13197-014-1666-2

Salvetti, E., Torriani, S., Felis, G.E., 2012. The genus *Lactobacillus*: a taxonomic update. *Probiotics Antimicrob. Prot.* 4, 217-226. https://doi.org/10.1007/s12602-012-9117-8

Sarika, A.R., Lipton, A.P., Aishwarya, M.S., Dhivya, R.S., 2012. Isolation of a bacteriocin-producing *Lactococcus lactis* and application of its bacteriocin to manage spoilage bacteria in high-value marine fish under different storage temperatures. *Appl. Biochem. Biotechnol.* 167, 1280-1289. https://doi.org/10.1007/s12010-012-9701-0

Sharmila, P.S., Vidya, A.K., 2015. Characterization and antibacterial activity of bacteriocin-producing *Bacillus subtilis* isolated from raw milk. *Int. J. Appl. Bioengineering.* 9, 1-6. https://doi.org/10.18000/ijabeg.10129

Subagiyo, S., Margino, S., Triyanto, T., 2015. Pengaruh penambahan berbagai jenis sumber karbon, nitrogen dan fosforpada medium deMan Rogosa and Sharpe (MRS) terhadap pertumbuhan bakteri asam laktat terpilih yang diisolasi dari intestinum udang penaeid. *J. Kelautan Tropis.* 18, 127-132.

Todorov, S.D., Prevost, H., Lebois, M., Dousset, X., LeBlanc, J.G., Franco, B.D.G.M., 2011. Bacteriocinogenic *Lactobacillus plantarum* ST16Pa isolated from papaya (*Carica papaya*)-from isolation to application: characterization of a bacteriocin. *Food Res. Int.* 44, 1351-1363. https://doi.org/10.1016/j.foodres.2011.01.027

Tomusiak-Plebanek, A., Heczko, P., Skowron, B., Baranowska, A., Okoń, K., Thor, P.J., Strus, M., 2018. *Lactobacilli* with superoxide dismutase-like or catalase activity are more effective in alleviating inflammation in an inflammatory bowel disease mouse model. *Drug Des. Devel. Ther.* 12, 3221-3233. https://doi.org/10.2147/DDDT.S164559.

Voulgari, K., Hatzikamari, M., Delepoglou, A., Georgakopoulos, P., Litopoulou-Tzanetaki, E., Tzanetakis, N., 2010. antifungal activity of non-starter lactic acid bacteria isolates from dairy products. *Food Control.* 21, 136–142. https://doi.org/10.1016/j.foodcont.2009.04.007

[WHO] World Health Organization. 2018. Salmonella (non-typhoidal). Available at: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal). [Date accessed: 29 December 2022]

Yanagida, F., Chen, Y.S., Shinohara, T., 2006. Searching for bacteriocin-producing lactic acid bacteria in the soil. *J. Gen. Appl. Microbiol.* 52, 21-28. https://doi.org/10.2323/jgam.52.21

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, 48. https://doi.org/10.1186/2191-0855-2-48

Yeni, Y., Aulia, R., Handayani, T., 2017. Keberadaan dan multiresistensi antibiotik *Salmonella* spp. dari produk perikanan segar di wilayah DKI Jakarta dan Bogor. *JPB Kelautan dan Perikanan.* 12, 79-90. https://doi.org/10.15578/jpbkp.v12i1.345

Zarrazquin, I., Torres-Unda, J., Ruiz, F., Irazusta, J., Kortajarena, M., Hoyos, C.I., Gil, J., Irazusta, A., 2014. Longitudinal study: lifestyle and cardiovascular health in health science students. *Nutr. Hosp.* 30, 1144-1151. https://doi.org/10.3305/nh.2014.30.5.7833.

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*. *Int. J. Syst. Evol. Microbiol.* 70, 2782-2858. https://doi.org/10.1099/ijsem.0.004107