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Characterisation of a bacteriocin produced by *Lactobacillus rhamnosus* IN13 isolated from *inasua*, a fermented fish product from Central Maluku, Indonesia

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Abstract

Lactobacillus rhamnosus IN13, a bacteriocin-producing bacterium, was isolated from *inasua*, a fermented fish product originated from Central Maluku, Indonesia. Bacteriocin produced by *Lactobacillus rhamnosus* IN13 could inhibit the growth of Gram-positive bacterium *Listeria monocytogenes* ATCC 7644 and Gram-negative bacterium *Salmonella* Typhymurium ATCC 14028. The bacteriocin was found to be heat stable at 121°C for 15 min. Moreover, it could maintain full stability after 30 d of storage at -40, -20, and 4°C. It was also stable at pH 2.0 – 10.0. The bacteriocin activity is proteinaceous in nature, since it could be inactivated by proteolytic enzymes. Molecular weight of the bacteriocin was 35 kDa. The present work showed that the bacteriocin produced by *L. rhamnosus* IN13 has potentials in food safety due to its ability in inhibiting Gram-positive and Gram-negative pathogens; thus, it may have future applications in food preservation.

Introduction

Bacteriocin could inhibit the growth of Gram-positive and Gram-negative bacteria, and has been used in some countries as a natural food preservative (Chikindas *et al.*, 2018). Bacteriocin is non-toxic, digestible by the digestive enzymes, non-harmful for intestinal microbes, and stable at various pH's and temperatures (Cleveland *et al.*, 2001). Lactic acid bacteria (LAB) are a group of bacteria that can produce bacteriocin with a synthesis pattern like that of protein synthesis. The bacteriocin synthesis is regulated by bacteriocin-coding gene. Bacteriocin has various molecular weights and biochemical characteristics (Drider *et al.*, 2006). The working mechanism of a bacteriocin depends on its concentration, ionisation ability, temperature and pH. Therefore, the use of bacteriocin as a natural preservative should consider these characteristics, since food processing involves various methods such as high or low processing temperature, drying, cooling and long storage period.

Several studies have reported that LAB isolated from fermented foods can produce bacteriocin, such as *Weissella cibaria* 110 that was isolated from '*plaa-som*', a Thai fermented fish product, which can inhibit the growth of Gram-positive bacteria (Srionnual *et al.*, 2007). *Staphylococcus homini* KQU-131 that was isolated from '*pla-ra*', a Thai marine fish fermented product, produced a bacteriocin that is stable at high temperatures. *Lactobacillus plantarum* PMU33 that was isolated from '*som-fak*', a Thai fermented fish product, produced bacteriocin and inhibited the growth of *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* (Noonpakdee *et al.*, 2009).

Lactic acid bacteria isolated from '*bekasam*', a fermented food from Indonesia, can produce bacteriocin and inhibit the growth of food pathogenic bacteria (Desniar *et al.*, 2011). Other studies in Indonesia have also isolated LAB from a fermented fish product from Kalimantan. The isolate was identified as *Pediococcus pentosaceus* that could produce bacteriocin and inhibit the growth of *S. aureus* (Kusmarwati *et al.*, 2014).

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Based on those studies, it is highly possible to find bacteriocin-producing bacteria in other fermented fish products, such as *inasua*. *Inasua* is made from various types of fish, especially marine fish, such as *Hoplacrchus psittacus*, *Terapon jarbua* and *Caranx ignobilis*. *Inasua* is usually sold as fish filet, and has a distinctive aroma of fermented product and salty taste. *Inasua* is prepared with salt concentration of 20 - 30% and 'nira', with the fermentation process performed in closed container at room temperature for three months (Nendissa, 2001). Properly processed inasua can last up to a year; however, there have been no published reports on the compounds produced by bacteria involved in the fermentation of this product. Mahulette *et al.* (2016) has isolated LAB from *inasua* and the isolate was identified as *Lactobacillus rhamnosus* IN13, but unfortunately, its bacteriocin has not been characterised. Thus, the aims of the present work were to determine the growth and production curve of *L. rhamnosus* IN13; to determine the LAB bacteriocin's molecular weight; and to characterise the LAB bacteriocin such as the effect of temperature, NaCl, surfactant, and pH on the bacteriocin activity.

Materials and methods

Production of bacteriocin

Lactobacillus rhamnosus IN13 was cultivated on De Man, Rogosa and Sharpe (MRS) agar added with 0.5% of $CaCO_3$ and 3% of NaCl, incubated anaerobically at 37°C for 24 h, and the supernatant was harvested from 1 mL of culture that was centrifuged at 6,000 *g* for 10 min. The pH of the cell-free supernatant was adjusted to pH 6.5 using 1 M NaOH to prevent the inhibitory effect of organic acids. The supernatant was then filtered using a 0.22 µm membrane filter (Millipore). The filtrate collected was used for bacteriocin characterisation (Ogunbanwo *et al.*, 2003).

Determination of bacteriocin activities

Well diffusion agar method was used to examine the antimicrobial activity of the extracted bacteriocin (Yanagida *et al.*, 2005). Briefly, 100 µL of bacterial cultures, i.e., *Salmonella Typhymurium* and *Listeria monocytogenes* (10^6 CFU/mL), were inoculated on nutrient agar (NA). Next, 50 µL of the bacteriocin was put into a well with a diameter of 8 mm made on the NA medium that had been inoculated with the bacterial strains. The cultures were then incubated at 37°C for 24 h. Then, the diameter of the clear zone formed on the medium following incubation was measured with a calliper and calculated using a mathematical equation (Sharma *et al.*, 2006). The bacteriocin inhibitory activity against the indicator bacteria was expressed as AU (activity unit). One AU/mL is the area of inhibition area per unit volume of bacteriocin tested (Ivanova *et al.*, 2000).

$$\text{The activity of bacteriocin (AU/mL)} = Lz - Ls/V \quad \text{(Equation 1)}$$

where Lz = area of clear zone (mm^2), Ls = area of well (mm^2), and V = volume of samples (mL).

Determination of growth curve and bacteriocin production

Lactobacillus rhamnosus IN13 was grown in 200 mL MRS broth and incubated at 37°C for 48 h. One mL of culture was taken every 3 h and put in a test tube. Changes in the optical density of the culture were recorded at 600 nm. Moreover, it was also tested for its bacteriocin activity against indicator bacteria and its protein content was calculated (Lisboa *et al.*, 2006).

Precipitation of bacteriocin

Precipitation of bacteriocin was done by using Scopes method (Scopes, 1994) with ammonium sulphate concentration of 60%. The purified bacteriocin was stored at 4°C for 24 h, and then centrifuged at 6,000 for 40 min. The precipitate formed was dissolved in 0.1 M phosphate buffer at pH 7.0 with volume ± 2 mL (Lisboa *et al.*, 2006). Then antimicrobial activity was examined.

Molecular weight determination

Molecular weight of the bacteriocin was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Schägger and von Jagow, 1987) with vertical slab gel system (Bangalore Genei, India) with 4% of stacking gel and 12% of separating gel. The electrophoresis was performed at 50 mA and 100 V for 40 min. Following completion of the run, the gel was stained with Coomassie brilliant blue G-250, and the molecular weight of bacteriocin was calculated from the relative mobility of molecular weight markers.

Bacteriocin sensitivity of the Proteinase K assay

The sensitivity assay of proteolytic enzymes was performed by adding 250 µL of Proteinase K (1 mg/mL) in 250 µL of cell-free supernatant and homogenised, then it was incubated for 2 h at 37°C (Kusmarwati *et al.*, 2013) before it was further tested

for its inhibitory activity against the indicator bacteria. A negative result suggests that the bacteriocin is a proteinaceous compound (Savadogo *et al.*, 2006)

Determination of temperature effect

The assay was performed on several temperatures (*i.e.*, high, low, freezing). The bacteriocin stability at high temperature was carried out with 1.5 mL of bacteriocin that was incubated at 60, 70, 80 and 100°C for 30 min, 121°C for 15 min, and 121°C for 5 min, with untreated bacteriocin served as control. Similar tests for low and freezing temperatures were performed with 1.5 mL of bacteriocin that was stored at 4°C, -20°C and -40°C for 4 w, and the antimicrobial activity was then examined (Sharma *et al.*, 2006).

Determination of NaCl effect

The assay was performed on several NaCl concentrations (*i.e.*, 2 - 10%), where NaCl was introduced into 1 mL of bacteriocin. Untreated sample served as control. The samples and control were incubated at 37°C for 120 min, and the antimicrobial activity was then examined (Adinarayana *et al.*, 2002).

Determination of surfactant effect

The assay was performed on two types of surfactants (*i.e.*, SDS, Tween 80) where 0.1 mL or 0.01 g of surfactant was introduced into 1 mL of bacteriocin. Untreated sample served as control. The samples and control were incubated at 37°C for 60 min, and the antimicrobial activity was then examined (Kusmarwati *et al.*, 2013).

Determination of pH effect

The effect of pH on bacteriocin activity was tested using a suspension of bacteriocin extract in 50 mM citrate buffer of pH 2.0 and 3.0 in 50 mM potassium phosphate buffer of pH 4.0, 5.0, 6.0, 7.0, and 8.0, 50 mM glycine NaOH buffer of pH 9.0, 10.0, and 11.0. Untreated sample served as control. The samples and control were incubated at 30°C for 4 h. After the incubation was complete, the suspension of bacteriocin was neutralised with HCl and NaOH (Larsen *et al.*, 1993), and the antimicrobial activity was then examined (Yanagida *et al.*, 2005).

Results and discussion

Growth and production curve of bacteriocin

Lactobacillus rhamnosus IN13 could grow well on MRSA by adding 0.5% $CaCO_3$ and 3% NaCl at pH 7.0 and incubated at 37°C for 24 - 48 h (Figure 1). Srinivasan *et al.* (2013) reported that *L. rhamnosus*

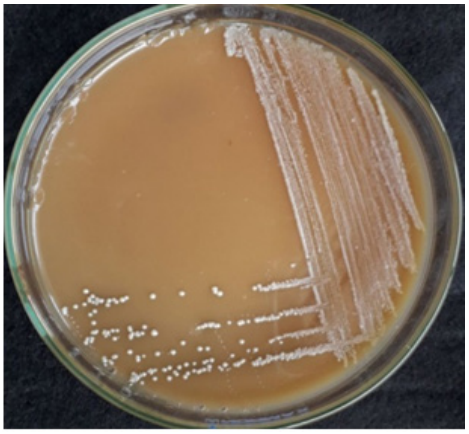

Figure 1. *Lactobacillus rhamnosus* IN13 on MRSA medium with 0.5% CaCo3 addition, 3% NaCl pH 7.0 condition, incubated at 37°C for 24 – 48 h.

L34 that was isolated from various types of animal milk could grow well on MRSA following incubation at 37°C for 24 - 48 h. The isolate was able to produce bacteriocin, which inhibited the growth of some pathogenic bacteria i.e., *Listeria monocytogenes* MTCC 1143 and *Listeria monocytogenes* MTCC 9806. MRS medium contained nitrogen and carbon as a source of nutrients for bacteria to grow. *Lactobacillus rhamnosus* IN13 produced antimicrobial compounds and inhibited the growth of *Salmonella* Typhymurium ATCC 14028 and *Listeria monocytogenes* ATCC 7644, of which both bacteria are the cause of the foodborne diseases known as salmonellosis and listeriosis, respectively (Rivoal *et al.*, 2010). Listeriosis is caused by *L. monocytogenes* and it has mortality rate of about 20 - 30%. *L. monocytogenes* is present in various environments, and it is heat, acid, and salt resistant. Moreover, these bacteria can grow at 4°C and form biofilms (Esteban *et al.*, 2009).

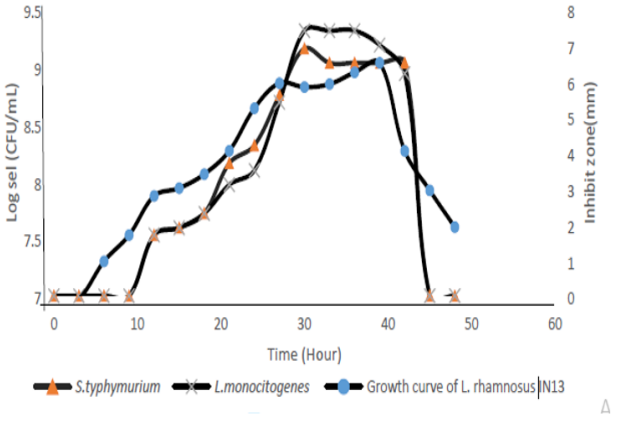

Figure 2. The bacterial growth curve of *Lactobacillus Rhamnosus* IN13 and bacteriocin activity against bacterial inhibition of *Listeria monocytogenes* and *Salmonella* Typhymurium.

Based on the inhibition zone formed, bacteriocin started to be produced after 12 h of incubation, and it reached its optimum production at 30 h. The antimicrobial activity remained similar at 30 - 33 h of growth. However, the bacteriocin activity started to decrease after 40 h (Figure 2). Kusmarwati *et al.*, (2014) successfully isolated LAB from '*rusip*', a traditional dish from Bangka, that could produce bacteriocin at 37°C after 24 - 48 h. Another study showed that *L. rhamnosus* isolated from grape skin had bacteriocin activity in the exponential and stationary phases, but after that, there was no more bacteriocin activity detected (Mourad *et al.*, 2005).

Molecular size of bacteriocin

The bacteriocin produced by the *L. rhamnosus* IN13 showed inhibition against *L. monocytogenes* and *S.* Typhymurium. The inhibition zone of the extract was precipitated with ammonium sulphate. The molecular weight of the bacteriocin produced by L. rhamnosus IN13 was estimated to be 35 kDa (Figure 3). Riley *et al.* (2002) reported that the molecular weight of bacteriocin produced by *Lactobacillus* sp. ranged from 40 to 80 kDa, while the bacteriocin produced by *L. lactis* varied around 94 kDa. Those studies showed that bacteriocin produced from different bacterial species has different molecular weights and their activity is different under various environmental conditions. Bacteriocin is classified into four groups based on its biochemical, structural, and genetic characteristics: Class I (lantibiotics), Class II (non-lantibiotics), Class III (peptides with large molecular weight of > 30 kDa), and Class IV (bacteriocin-protein complex) (Cotter *et al.*, 2005).

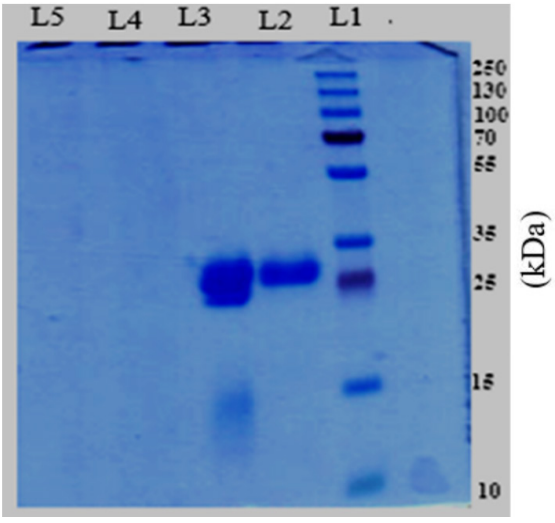

Figure 3. SDS-PAGE analysis of bacteriocin from *Lactobacillus rhamnosus* IN13. L1: protein marker; L2: precipitated bacteriocin; L3: cell-free supernatant; L4: precipitated bacteriocin with addition of Proteinase K; L5: cell-free supernatant with an addition of Proteinase K.

Based on the molecular weight, the bacteriocin produced by *L. rhamnosus* IN13 in the present work could be categorised as Class III. According to Parada *et al.* (2007), bacteriocin of Class III is a peptide with a large molecular weight more than 30 kDa and resistant to heat, such as helviticin J, and lactacin A and B.

Characteristics of bacteriocin

The antimicrobial activity of purified bacteriocin produced by *L. rhamnosus* IN13 was examined for its sensitivity to Proteinase K. Under the condition tested, the activity of the bacteriocin was found to be eliminated by Proteinase K. The reduction of activity by proteolytic enzymes suggested that the antimicrobial substance was not lipid but proteinaceous in nature. The bacteriocin activity was categorised to be extremely heat stable since there was no decrease in activity after incubation for 15 min at 121°C. Similarly, the bacteriocin was still active after being kept at -40°C for 4 w (Figure 4). The antibacterial activity of bacteriocin produced by *L. rhamnosus* IN13 was highly resistant to heat, acid, and alkali treatments. Heat stability is a major characteristic of some bacteriocins produced by the LAB, with the temperature ranges from 60°C to 100°C for more than 30 min, such as shown by lactosin 27, lactosin S, carnobacteriocins A and B, lactacin B, lactacin F, and nisin that are resistant to autoclaving temperature for 15-20 min (De Vuyst and Vandamme, 1994). Other studies have shown that bacteriocin is stable to heat after incubation at 100°C for 15 min (Srinivasan *et al.*, 2013). Based on its characteristics, the bacteriocin of *L. rhamnosus* IN13 showed potentials to be used as preservatives in processed foods produced at high temperature (Gao *et al.*, 2010).

The stability of the bacteriocin was also determined at different salt concentrations ranging between 2.0 to 10%. The results showed that the bacteriocin activity was stable at concentration 2 - 10% of salt (data not shown). Kusumawati (2000) reported that there is no effect of bacteriocin activity by addition of 0 - 10% NaCl, which was seen in the present work from the comparison with control.

The bactericidal activity was also tested for the effect of surfactant using SDS and Tween 80, which showed that the bacteriocin remained stable after the addition of surfactants (Figure 5). Xie *et al.* (2009) reported that *Bacillus subtilis* was able to produce bacteriocin whose activity did not decrease after the addition of surfactant. Other studies have also reported that the addition of chemicals to bacteriocin did not cause any effect on its activity as shown by a

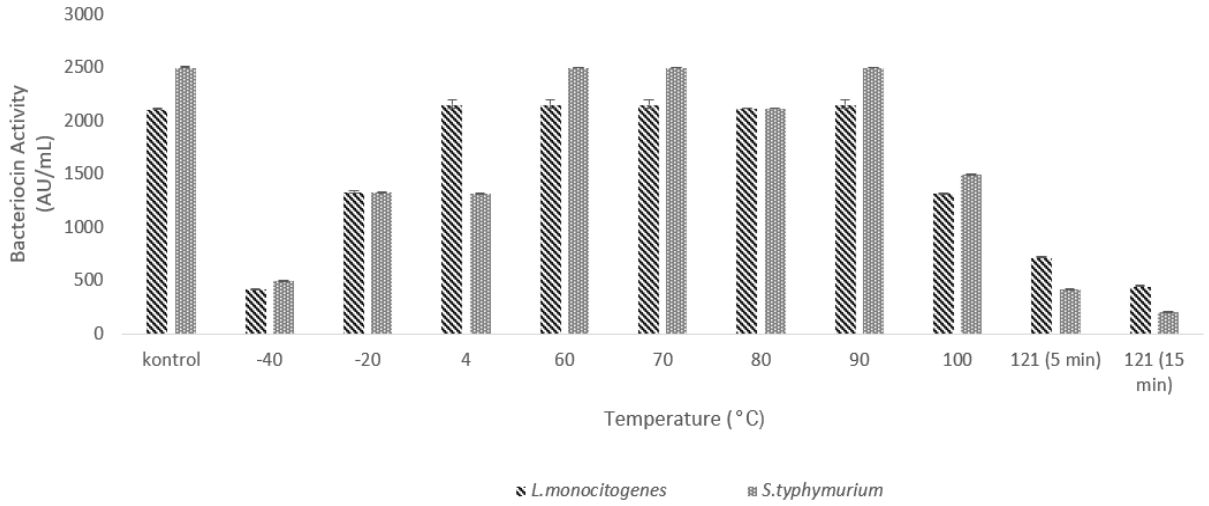

Figure 4. Effect of temperatures on bacteriocin activity against *Salmonella* Typhymurium and *Listeria monocytogenes*.

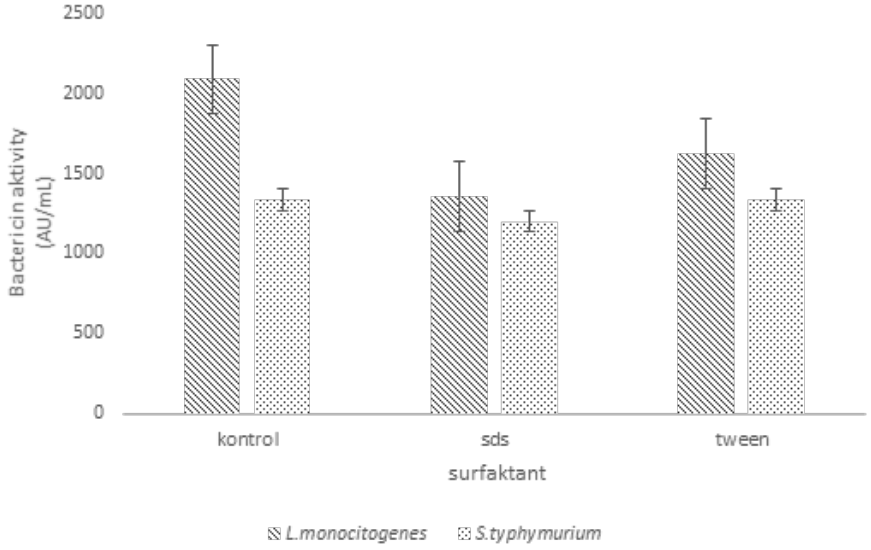

Figure 5. Effect of surfactants on bacteriocin activity against *Salmonella* Typhymurium and *Listeria monocytogenes.*

study using Tween 20, Tween 80, urea, Triton X-100, and Triton X-114 that were added into the bacteriocin produced by R1333, *L. sakei* ACU and *L plantaricin* C19, where the surfactants were found to have no effect on the bacteriocin activity (Castro *et al.*, 2011)

The results also showed that the bacteriocin had good activity at a low pH of 2.0 to 10.0. However, the antimicrobial activity decreased at pH 11.0 (data not shown). Srinivasan *et al.* (2013) found that bacteriocin from *L. rhamnosus* L34 could survive at pH 2.0 – 8.0 after 24 h of incubation. Another study showed that bacteriocin R1333 and ST16 remained stable at pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0, while a bacteriocin produced by *L. brevis* OG1 was stable at pH 2.0 to 8.0 (Ogunbanwo *et al.*, 2003).

Conclusion

Lactobacillus rhamnosus IN13 isolated from *inasua*, a fermented fish product from Central Maluku, Indonesia was the bacterium found to be capable of producing bacteriocin that inhibited the growth of *Salmonella* Typhymurium and *Listeria monocytogenes*. The bacteriocin also showed a heat-resistant characteristic following incubation for 15 min at 121°C, and it was also resistant to cold temperature after incubation for 4 w at -40°C. Moreover, it was stable at pH 2.0 – 10.0 and various concentrations of NaCl did not affect the bacteriocin activity. Furthermore, the bacteriocin also showed good stability against several types of surfactants.

The present work has shown that the bacteriocin produced by *L. rhamnosus* IN13 isolated from *inasua* to have the potentials to be used as food preservative.

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References

Adinarayana, K. and Elllaiah, P. 2002. Response surface optimization of the critica medium components for the production of the alkaline protease by newly isolated *Bacillus sp.* Journal of Pharmacy and Pharmaceutical Sciences 5(3): 272-278.

Castro, M. P., Palavecino, N. Z., Herman, C., Garro, O. A. and Campos, C. A. 2011. Lactic acid bacteria isolated from artisanal dry sausages: characterization of antibacterial compounds and study of the factors affecting bacteriocin production. Meat Science 87(4): 321-329.

Chikindas, M. L., Weeks, R., Drider, D., Chistyakov, V. A. and Dics, L. M. 2018. Function and emerging applications of bacteriocins. Current Opinion in Biotechnology 49: 23-28.

Cleveland, J., Montville, T. J., Nes, I. F. and Chikindas, M. L. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 71(1): 1-20.

Cotter, P. D., Hill, C. and Rossa, R. P. 2005. Bacteriocins: developing innate immunity for food. Nature Reviews Microbiology 3(10): 777-788.

Desniar, Rusmana, I., Suwanto, A. and Mubarik, N. R. 2011. Screening for bacteriocin of lactic acid bacteria from bekasam. Jurnal Pengolahan Hasil Perikanan Indonesia 14(2): 124-133.

Drider, D., Fimland, G., Héchard, Y., McMullen, L. M. and Prévost, H. 2006. The continuing story of class IIa bacteriocins. Microbiology and Molecular Biology Reviews 70(2): 564-582.

Esteban, J. I., Oporteo, B., Aduariz, G., Justir, R. A. and Hurtado, A., 2009. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. BMC Veterinary Research 5: article ID 2.

Gao, Y., Jia, S., Gao, Q. and Tan, Z. 2010. A novel bacteriocin with broad inhibitory spectrum produced by *Lactobacillus sake* C2, isolated from traditional Chinese fermented cabbage. Food Control 21(1): 76-81.

Ivanova, I., Kabadjova, P., Pantev, A., Danova, S. and Dousset, X. 2000. Detection, purification and partial characterization of a novel bacteriocin substance produced by *Lactococcus lactis* subsp. lactis b14 isolated from Boza - Bulgarian traditional cereal beverage. Biocatalysis: Fundamentals and Applications 41(6): 47-53.

Kusmarwati, A., Arief, F. R. And Haryati, S. 2014. Exploration of bacteriocin from lactic acid bacteria origin from Bangkanese and Kalimantanese rusip. JPB Perikanan 9(1): 29-40.

Kusmarwati, A., Indriati, N. and Hermana, I. 2013. Production and characterization bacteriocin produced by lactic acid bacteria isolated from rusip. Squalen 8(1): 13-22.

Kusumawati, N. 2000. Peranan bakteri asam laktat dalam menghambat bakteri *Listeria monocitogenes* pada bahan pangan. Jurnal Teknologi Pangan dan Gizi 1(1): 14-28.

Larsen, A. G., Vogensen, F. K. And Josephsen, J. 1993. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401. Journal of Applied Bacteriology 75: 113-122.

Lisboa, M. P., Bonatto, D., Bizani, D., Henriques, J. A. and Brandelli, A. 2006. Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic forest. International Microbiology 9(2): 111-118.

Mahulette, F., Mubarik, N. R., Suwanto, A. and Widanarni. 2016. Isolation and characterization of lactic acid bacteria from *inasua*. Journal of Tropical Biodiversity and Biotechnology 1(2): 71-76.

Mourad, K., Halima, Z.-K. and Nour-Eddine, K. 2005. Detection and activity of plantaricin OL15 a bacteriocin produced by *Lactobacillus Plantarum* OL 15 isolated from Algerian fermented olive. Grasas y Aceites 56(3): 192-197.

Nendissa, S. J. 2001. Pemanfaatan kultur *Pedioccocus acidilactici* F 11 penghasil bakteriosin untuk memperbaiki kualitas *inasua* (ikan asin) gurame (*Osphoremus gouramy* Lacepede). Yogyakarta: Universitas Gajah Mada, Unpublished MSc Thesis.

Noonpakdee, W., Jumriangrit, P., Wittayakom, K., Zendo J., Nakayama, J., Sonomoto, K. and Panyim, S. 2009. Two-peptide bacteriocin from Lactobacillus plantarum PMU 33 galur isolated from *som-fak*, a Thai low salt fermented fish product. Asia-Pacific Journal of Molecular Biology and Biotechnology 17(1): 19-25.

Ogunbanwo, S. T., Sanni, A. I. and Onilude, A. A. 2003. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African Journal of Biotechnology 2(8): 219-227.

Parada, J. L., Caron, C. R., Medeiros, A. B. and Soccol, C. R. 2007. Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Brazilian Archives of Biology and Technology 50(3): 512-542.

Riley, M. A. and Wertz, J. E. 2002. Bacteriocins: evolution, ecology and application. Annual Review of Microbiology 56: 117-137.

Rivoal, K., Quéguiner, S., Boscher, E., Bougeard, S., Ermel, G., Salvat, G., ... and Protais, J. 2010. Detection of *Listeria monocytogenes* in raw and pasteurized liquid whole eggs and characterization by PFGE. International Journal of Food Microbiology 138(1-2): 56-62.

Savadogo, A., Cheik Ouattara, A. T., Imael Bassole, H. N. and Alfred Traore, S. 2006. Bacteriocins and lactic acid bacteria - a minireview. African Journal of Biotechnology 5(9): 678-683.

Schägger, H. and von Jagow, G. 1987. Tricine-sodium dodecyl sulphate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analytical Biochemistry 166(2): 368-379.

Scopes, R. K. 1994. Protein purification - principles and practice. 3rd ed. New York: Springer Verlag.

Sharma, N., Kapoor, G. and Neopaney, B. 2006. Characterization of a new bacteriocin produced from a novel isolated galur of *Bacillus lentus* NG121. Antonie Van Leeuwenhoek 89(3-4): 337-343.

Srinivasan, R., Kumawat, D. K., Kumar, S. and Saxena, A. K. 2013. Purification and characterization of bacteriocin from *Lactobacillus rhamnosus* L34. Annals of Microbiology 63(1): 387-392.

Srionnual, S., Yanagida, F., Lin, L.-H., Hsiao, K.-N. and Chen, Y.-S. 2007. Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110 isolated from Plaa-Som, a fermented fish product from Thailand. Applied and Environmental Microbiology 73(7): 2247-2250.

Vuyst, L. D. and Vandamme, E. J. 1994. Lactic acid bacteria and bacteriocins: their practical importance. London: Chapman and Hall.

Xie, J., Zhang, R., Shang, C, and Guo, Y. 2009. Isolation and characterization of a bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits antimicrobial activity against domestic animal pathogens. African Journal of Biotechnology 8(20): 5611-5619.

Yanagida, F., Chen, Y., Onda, T. and Shinohara, T. 2005. Durancin L28- 1A, a new bacteriocin from *Enterococcus durans* L28-1, isolated from soil. Letters in Applied Microbiology 40(6): 430-435.