PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2017.299.305

Research Article
Effect of the Bacteriocin-producing *Bacillus cereus* Strain HVR22 on the Preservation of Fish Fillets

Yusra and Yempita Efendi

Department of Fisheries and Marine Sciences, Bung Hatta University, 25133 Padang, Indonesia

Abstract

Background and Objective: The *Bacillus cereus strain* HVR22 bacteria was obtained from isolated budu which produces bacteriocins. The aim of this study was to analyze the effect of crude bacteriocins on pathogen bacteria to observe the effect on cellular morphology of *Escherichia coli* using electron microscopy and to evaluate its effect on fish fillets. **Materials and Methods:** A diffusion method was used to measure the width of the clear zone made by *Escherichia coli*. Antimicrobial solutions of the *Bacillus cereus* strain HVR22 microbes were used to soak the snapper fillets, bacterial counts were performed on day 0 and 7. **Results:** Changes in *E. coli* membrane cell structure were observed under Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) after 48 h. Membranes were exposed to antimicrobial compounds from the *Bacillus cereus* strain HVR22. Antimicrobials produced by the *Bacillus cereus* strain HVR22 inhibited *E. coli* and *Salmonella* sp., growth on day 0 and 7. **Conclusion:** The bacteriocin-producing *Bacillus cereus* strain HVR22 could be used as a natural preservative for fish products.

Key words: *Bacillus cereus*, bacteriocins, application, fillet

Received: December 02, 2016 Accepted: March 13, 2017 Published: April 15, 2017

Citation: Yusra and Yempita Efendi, 2017. Effect of the bacteriocin-producing *Bacillus cereus* strain HVR22 on the preservation of fish fillets. Pak. J. Nutr., 16: 299-305.

Corresponding Author: Yusra, Department of Fisheries and Marine Sciences, Bung Hatta University, 25133 Padang, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The *Bacillus cereus* strain HVR22 is an isolate from budu which has antimicrobial activity toward *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Listeria monocytogenes* bacteria. Budu is a traditional fish-fermented product from West Sumatra that is usually made from pelagis fish tenggiri (*Scomberomorus* sp.) and talang-talang (*Chorinemus* sp.)[1]. The fermentation product is made without additional starter (called spontaneous fermentation) over 30 h and then gutted, salted and dried. Lactic Acid Bacteria (LAB) is usually produced from the fish fermented product.

The LAB is a fermented bacteria that acts as biopreservative because it can produce antimicrobial compounds, such as lactic acid, hydrogen peroxide (H_2O_2), diacetyl, carbon dioxide (CO_2) and bacteriocins[2,3]. Bacteriocins are polypeptide compounds that act as antimicrobial compounds[4]. Bacteriocins can be used as a starter culture that is produced through the fermentation process as a component of the food probiotic and as biopreservative, extending the shelf life of different food products[5,6]. Using LAB in food processing can reduce the use of chemical preservatives and can improve food palatability and nutritional quality by increasing protein and vitamins. The LAB also produces detoxifying agents and inhibitory compounds, such as CO_2, organic acids, ethanol, hydrogen peroxides and bacteriocins[7-10].

Bacteriocins are produced by prokaryotic and eukaryotic organisms[11], proteinaceous compounds which have antagonistic effects against related species and against spoilage and food-borne pathogens[12-14]. These peptides are very important for food and fodder, since a sufficient amount of peptides can inhibitor kill pathogenic microorganisms that compete for the same nutritive demands[6]. Bacteriocins can be produced by gram-positive and gram-negative bacteria[15,16]. *Bacillus* sp., *B. subtilis*, *B. thuringiensis*, *B. stearothermophilus*, *B. licheniformis*, *B. megaterium*, *B. thermoleovorans*, *B. cereus* and *B. coagulans* can also produce bacteriocins. Colicin, warnerin and coagulin are bacteriocins produced by *Escherichia coli*, *Staphylococcus warneri* (FM10, FM20 and FM30) and *Bacillus coagulan*, respectively[16]. Bacteriocins from LAB have drawn considerable attention recently because they are "Generally Recognized as Safe (GRAS)" and may be used as biopreservatives in food processing[17,18]. Different types of bacteriocins are widely used in the food industry, including niacin from *Lactococcus lactis* subsp. *lactis* and pediocin from *Pediococcus pentosaceus*[19,20]. Different strains of enterococci can produce active enterocins against *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium* spp.[21,22].

Bacteriocin producing LABs can be isolated from different types of food products such as dairy products, sausages, fish and vegetables[23-27]. Many Sudanese products are rich in LAB and can be used as biopreservatives. Different strains of LAB have been recorded as part of the process of producing traditional fermented milk products but the knowledge of their health benefits and species properties require further study[28]. Sudanese dairy products may be a rich source of LAB[29]. One study reported that the dominant species of LAB include *Lactobacillus xylosus*, *Lactococcus lactis* sub. sp. *cremoris*, *Lactobacillus delbrueckii* and *Pediococcus cerevisiae*. Several other studies on different Sudanese products (meat, fish, dairy, vegetables and cereal products) reported on their microbial load, however these products require additional research. This study was conducted to evaluate the effect of partial purification of bacteriocins on pathogen bacteria and to observe of its effect on the cellular morphology of *Escherichia coli* with applications to fish fillets.

MATERIALS AND METHODS

Bacterial strains: The *Bacillus cereus* strain HVR22 was provided by Yusra[1]. The indicator strain used in this study was *Escherichia coli*, which was provided by the Laboratory of Clinical Microbiology Research, Faculty of Medicine and Microbiology, University of Indonesia.

Antagonistic activity of bacteriocins: Diffusion method on sterile paper tests were used to evaluate the antimicrobial activity of the extracted bacteriocins. This method uses a paper disc. The culture of the *Bacillus cereus* strain HVR22 was refreshed in MRSA medium for 48 h, then 1 oz of bacteria was added to 10 mL of MRS broth and was incubated in 37°C for 24 h. Following, the culture was inserted into eppendorf and was centrifuged at 6000 rpm for 10 min. A 0.1 mL smear of *Escherichia coli* was removed using sterile cotton. Then, 20 mL of extracted bacteriocin preparation (CBP) was placed in each paper disc and the plates were incubated in 37°C for 24 h to measure the diameter of the microbe free zone[30].

Scanning electron microscopy: The treated *Escherichia coli* cells exposed to bacteriocins of the *Bacillus cereus* strain HVR22 after incubation at 37°C for 48 h were examined by

SEM to visualize any morphological changes that occurred in the cell. The cell suspensions were fixed with 3% glutaraldehyde in Na-cacodylate buffer (100 mM, pH 7.1). The cells were pelleted, washed to remove glutaraldehyde and resuspended in the same buffer. A drop from each suspension was transferred to poly-L-lysine-treated silicon wafer chips, which were kept for 30 min in a hydrated chamber for the cells to adhere. The attached cells were post fixed by immersing the chips in 1% osmium tetroxide (OSO_4) in cacodylate buffer for 30 min, rinsed in the same buffer and dehydrated in ethanol in ascending concentrations (%) of: 50, 70, 95 (2x) and 100 (2x), for 10 min each. The chips were mounted on aluminium stubs and coated with gold-palladium in a sputter coater (Emitech K550, Ashford, Kent, England). The chips were viewed at 3 kV accelerating voltage in a Hitachi S-4000 field emission scanning electron microscope (JEM-JEOL JSM-5310LV type) and secondary electron images of cells for topography contrast were taken at several magnifications[31].

Transmission electron microscopy: The cell suspension of pathogenic bacteria exposed to bacteriocins from the *Bacillus cereus* strain HVR22 which was incubated in 37°C for 48 h, where harvested by centrifugation and washed twice with 0.1 M phosphate buffer (pH 7.3). The cells were fixed with 2.5% (v/v) glutaraldehyde and 2.0% (v/v) formaldehyde in 0.12 M phosphate buffer for 10 days and then post-fixed in 2% (w/v) osmium tetroxide in the same buffer for 45 min. The samples were dehydrated in a graded acetone series (30-100%) and embedding in Araldite-Durcupan for 72 h at 60°C. Thin sections (microtome UPC-20, Leica) were mounted on grids, covered with collodion film and post-stained with 2% uranyl acetate in Reynold's lead citrate. All preparations were observed with a tipe JEOL-1010[32].

Application of bacteriocins to fresh fish fillets: Crude bacteriocins were used to soak the sample. The bacteriocins were exposed by cutting the snapper fish fillet into approximately 10 g and soaking it in a bacteriocins for 30 min (the control was not soaked). After soaking the fillet was removed and drained. The fish fillet was packed in sterile HDPE plastic and labeled. All samples were stored between -2 and 0°C. Observations were made on day 0 and 7. Inspection of fillet quality was done by Total Plate Count (TPC) to determine the total number of *Coliform* and *Salmonella* bacteria in each sample.

RESULTS AND DISCUSSION

Antagonistic activity of bacteriocins: The *Bacillus cereus* strain HVR22 isolates had the highest inhibitory effect against the *Escherichia coli* in 14 mm as shown in Fig. 1[1]. Antimicrobial activity can be determined by the width of the clear zone around the paper disc after incubation for 48 h. The emergence of the clear zone is caused by acid metabolism from lactic acid that prevented growth of the indicator bacteria, the same effect was also found in Moroccan sardines (*Sardina pilchardus*) fermented fish. The width of the inhibitory zone of LAB was 20.6 mm and the bakasang fermentation inhibition zone diameter against *Escherichia coli* was 15 mm[33,34].

Scanning electron microscopy: The SEM revealed damage to the cells and cellular material loss. Cell morphological damage from *Escherichia coli* was used as a representative of gram negative cells, after treatment with the antimicrobial compounds from the *Bacillus cereus* strain HRV22. As shown in Fig. 2, damage to the *Escherichia coli* cell membrane resulted from interaction with the *Bacillus cereus* strain HRV22 from budu fish fermented product. The cell surface was wrinkled and rough and formed irregular shapes and there was a notch in a few cells with septa that had not yet split. Antibacterial bacteriocins disrupted the cell membrane target,

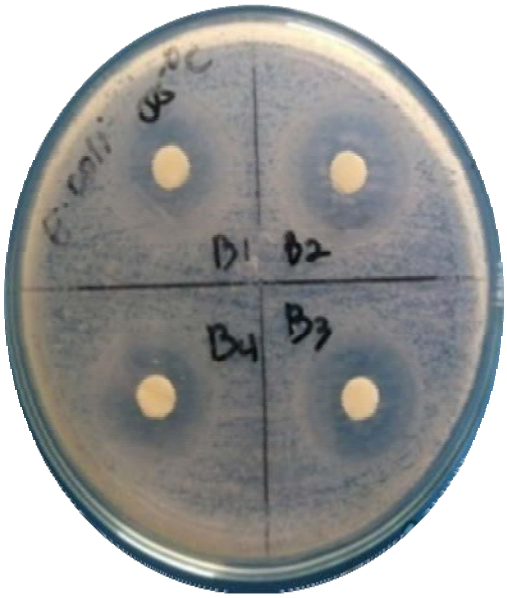

Fig. 1: Antimicrobial activity of isolate *Bacillus cereus* strain HVR22 on inhibiting the growth of *Escherichia coli* bacteria

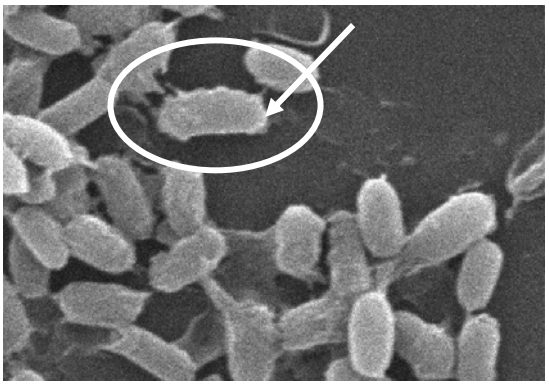

Fig. 2: Electron micrograph of *Escherichia coli* bacteria exposed to antibacterial of the *Bacillus cereus* strain HVR22 using Scanning Electronic Microscopy (SEM)

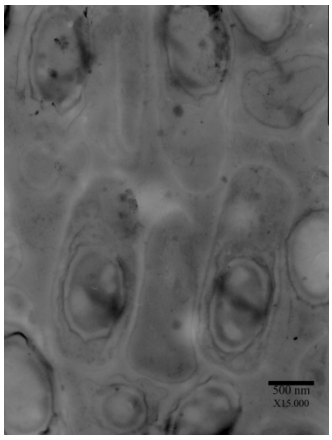

Fig. 3: Electron micrograph of *Escherichia coli* bacteria exposed to antibacterial of the *Bacillus cereus* strain HVR22 using Transmission Electronic Microscopy (TEM)

such that the cells lost their function[35]. Some bacteriocins cause bacteriolysis (lysis of bacterial cells) due to the deregulation of autolysis systems and damage to the peptidoglycan layer. Bacteriocins work at very low nanomolar concentrations[36].

Transmission electron microscopy: The TEM observation of ultra-structure pathogenic bacteria of the bacteriocins of *Bacillus cereus* strain HRV22 is shown in Fig. 3. The addition of bacteriocins to *Escherichia coli* pathogenic bacteria produced a few visible changes, including irregular cytoplasm, formation of a space between cytoplasm and cell membrane, wrinkled cells (collapse) and cell lysis. Based on microstructure, some *Escherichia coli* cells were broken and irregularly-shaped. Antimicrobial compounds produced by the *Bacillus cereus* strain HVR22 can damage the cytoplasm membrane and affect the integrity of *Escherichia coli* cell surface. This damage can cause increased permeability and intercellular discharge.

Cerein-8A activity analyzed against *Bacillus cereus* and *Listeria monocytogenes* bacteria using TEM showed that cerein-8A killed bacterial cells in 60 min[37]. Bacteriocins also inhibited some gram-positive bacteria, such as *Bacillus* spp., bacteria but these bacteriocins were not able to kill *Escherichia coli* and *Salmonella enteritidis* cells. Bacteriocins derived from *Bacillus cereus* SBS02 isolated from sea shells can inhibit the growth of pathogenic bacteria and bacteria that cause decay of food, such as *Candida albicans*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus subtilis*[38].

Electron microscopy showed cell lysis following treatment with antimicrobial compounds of the *Bacillus cereus* strain HVR22. Cell damage caused by antimicrobial compounds resemble that observed with crude bacteriocins[39]. Type-A1antibiotics, to which nisin, pediocin and apidermin and many other amphiphilic antimicrobial peptides belong, exert their activity by disrupting the functional barrier of microbial cytoplasmic membranes[40]. In addition to nisin and apidermin, lipid I and II binding has been observed in subtilin and mersacidin produced by *Bacillus* spp.[41]. The hydrophobic bacteriocin cerein 7, produced by *B. cereus* Bc7 has also been characterized as a membrane-active compound[42]. The antimicrobial compound from the *Bacillus cereus* strain HVR22 has a bactericidal effect caused from disrupting the membrane function of target organisms.

Application of bacteriocins to fresh fish fillets: Antibacterial compounds from the bacteria *Bacillus cereus* strain HVR22 can reduce the number of pathogenic bacteria contained in snapper fillets, including *Salmonella* sp. and *Escherichia* sp. bacteria, using PCA, SSA and EMBA medium as shown in Table 1. On observation day 0 using a PCA medium, the average colonies of bacteria were 6.1×10^8 CFU g^{-1} colonies. On observation day 7, bacteria were growing in the PCA but there were no *Salmonella* sp. and *Escherichia* sp. bacteria. Bacteriocins except in selective SSA and EMBA medium, as a result of the submersion of snapper fish fillet in crude bacteriocins from the *Bacillus cereus* strain HVR22 that contained antimicrobial compounds. Generally, bacteriocins oppose other bacteria through a bactericidal effect.

Red tilapia fillet submerged in 10^8 CFU g^{-1} liquid for 5, 10 and 15 min achieved a 9 day shelf life[43]. The addition of lactic acid bacteria (*Lactobacillus acidophilus*) can decrease the pH of tilapia (*Oreochromis niloticus*)[44]. *Lactobacillus fermentum* UN01 can extend the shelf life of fish in cold temperatures (refrigeration) up to 9 days[45]. Supernatant from cultivation

Table 1: Colonies of bacteria in treatment concentrations of crude bacteriocin in snapper fish fillet

		No. of colonies (CFU g^{-1})					
Treatments	Medium	Dilution 10^3 (day 0)	Dilution 10^5 (day 0)	Dilution 10^7 (day 0)	Dilution 10^3 (day 7)	Dilution 10^5 (day 7)	Dilution 10^7 (day 7)
Control	NA	142	91	56	96	47	6
	NA	140	90	66	77	10	5
	SSA	5	2	2	3	3	2
	SSA	4	2	1	4	3	2
	EMBA	4	1	2	6	2	2
	EMBA	2	1	1	3	3	1
Submersion in crude bacteriocin	NA	79	44	23	52	4	3
	NA	76	45	22	47	5	3
	SSA	3	2	2	0	0	0
	SSA	2	2	2	0	0	0
	EMBA	2	3	1	0	0	0
	EMBA	1	2	2	0	0	0

media of *L. plantarum* (SK5) can be used as a biopreservative for snapper fillets at chilling temperatures and can replace formaldehyde[46].

CONCLUSION

Antimicrobial compounds produced by the *Bacillus cereus* strain HVR22 can damage the cell membrane and cellular structures inside *Escherichia coli* cells. Antimicrobial compounds of the *Bacillus cereus* strain HVR22 can inhibit the growth of *Salmonella* sp. and *Escherichia* sp. in snapper fish fillet until 7 days of storage and can be used as natural preservatives.

SIGNIFICANCE STATEMENT

This study evaluated the effect of bacteriocins produced from the *Bacillus cereus* strain HVR22 and its application to fish fillet preservation. The researchers certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this study.

ACKNOWLEDGMENTS

This study was supported under study grant (Hibah Bersaing) Directorate of Research and Community Service, Ministry of Research Technology and Higher Education Republic of Indonesia [No.045-33/SP/HATTA-1/LPPM/IV-2015].

REFERENCES

1. Yusra, F. Azima, Novelina and Periadnadi, 2013. Antimicrobial activity of lactic acid bacteria isolated from Budu of West Sumatera to food biopreservatives. Pak. J. Nutr., 12: 628-635.
2. Ray, B., 2004. Fundamental Food Microbiology. 3rd Edn., CRC Press, Florida, ISBN-13: 9780849316104, Pages: 608.
3. Suardana, I.W., I.N. Suarsana, I.N. Sujaya and K.G. Wiryawan, 2007. Isolation and identification of acid lactic bacteria from Bali cattle's gastric fluid as a potential candidate of biopreservative. J. Vet., 8: 155-159.
4. Naidu, A.S and R.A. Clemens, 2000. Natural Food Antimicrobial Systems. CRC Press, Florida, ISBN: 9781420039368, Pages: 818.
5. Rahayu, E.S., 2010. Lactic acid bacteria and their role in food and health: Current research in Indonesia. Proceedings of the International Symposium of Lactic Acid Bacteria, July 25-27, 2010, Kuala Lumpur.
6. Deegan, L.H., P.D. Cotter, C. Hill and P. Ross, 2006. Bacteriocins: Biological tools for bio-preservation and shelf-life extension. Int. Dairy J., 16: 1058-1071.
7. Oyewole, O.B., 1997. Lactic fermented foods in Africa and their benefits. Food Control, 8: 289-297.
8. Holzapfel, W.H., 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. Int. J. Food Microbiol., 75: 197-212.
9. Hansen, E.B., 2002. Commercial bacterial starter cultures for fermented foods of the future. Int. J. Food Microbiol., 78: 119-131.
10. Chelule, P.K., M.P. Mokeona and N. Gqaleni, 2010. Advantages of Traditional Lactic Acid Bacteria Fermentation of Food in Africa. In: Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Mendez-Vilas, A. (Ed.). Formatex Research Center, Spain, pp: 1160-1167.
11. Papagianni, M., 2003. Ribosomally synthesized peptides with antimicrobial properties: Biosynthesis, structure, function and applications. Biotechnol. Adv., 21: 465-499.
12. Carolissen-Mackay, V., G. Arendse and J.W. Hastings, 1997. Purification of bacteriocins of lactic acid bacteria: Problems and pointers. Int. J. Food Microbiol., 34: 1-16.

13. Aymerich, M.T., M. Garriga, J.M. Monfort, I. Nes and M. Hugas, 2000. Bacteriocin-producing lactobacilli in Spanish-style fermented sausages: Characterization of bacteriocins. Food Microbiol., 17: 33-45.
14. Leroy, F. and L. De Vuyst, 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci. Technol., 15: 67-78.
15. De Vuyst, L. and E.J. Vandamme, 1994. Antimicrobial Potential of Lactic Acid Bacteria. In: Bacteriocins of Lactic Acid Bacteria, De Vuyst, L. and E.L. Vandamme (Eds.). Blackie Academic and Professional, Glasgow, United Kingdom, pp: 91-142.
16. Jack, R.W., J.R. Tagg and B. Ray, 1995. Bacteriocins of gram-positive bacteria. Microbiol. Rev., 59: 171-200.
17. Carr, F.J., D. Chill and N. Maida, 2002. The lactic acid bacteria: A literature survey. Crit. Rev. Microbiol., 28: 281-370.
18. Patil, M., A. Pal, V. Pal and R.K. Yaddula, 2007. Isolation of bacteriocinogenic lactic acid bacteria from rat intestine. J. Cult. Collect., 5: 58-63.
19. Rodriguez, J.M., 1996. Review: Antimicrobial spectrum, structure, properties and mode of action of nisin, a bacteriocin produced by *Lactococcus lactis*. Food Sci. Technol. Int., 2: 61-68.
20. Moreno, I., A.L.S. Lerayer, V.L.S. Baldini and M.F.D.F. Leitao, 2000. Characterization of bacteriocins produced by *Lactococcus lactis* strains. Braz. J. Microbiol., 31: 183-191.
21. Franz, C.M.A.P., W.H. Holzapfel and M.E. Stiles, 1999. Enterococci at the crossroads of food safety? Int. J. Food Microbiol., 47: 1-24.
22. Gelsomino, R., M. Vancanneyt, S. Condon, J. Swings and T.M. Cogan, 2001. Enterococcal diversity in the environment of an Irish Cheddar-type cheesemaking factory. Int. J. Food Microbiol., 71: 177-188.
23. Leroy, F., M.F. Moreno and L. De Vuyst, 2003. *Enterococcus faecium* RZS C5, an interesting bacteriocin producer to be used as a co-culture in food fermentation. Int. J. Food Microbiol., 88: 235-240.
24. Cintas, L.M., P. Casaus, L.S. Havarstein, P.E. Hernandez and I.F. Nes, 1997. Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. Applied Environ. Microbiol., 63: 4321-4330.
25. Herranz, C., P. Casaus, S. M ukhopadhyay, J.M. Martinez and J.M. Rodriguez *et al*., 2001. *Enterococcus faecium* P21: A strain occurring naturally in dry-fermented sausages producing the class II bacteriocins enterocin A and enterocin B. Food Microbiol., 18: 115-131.
26. Embarek, P.K.B., V.F. Jeppesen and H.H. Huss, 1994. Antibacterial potential of *Enterococcus faecium* strains isolated from sous-vide cooked fish fillets. Food Microbiol., 11: 525-536.
27. Bennik, M.H.J., B. Vanloo, R. Brasseur, L.G.M. Gorris and E.J. Smid, 1998. A novel bacteriocin with a YGNGV motif from vegetable-associated *Enterococcus mundtii*: Full characterization and interaction with target organisms. Biochim. Biophys. Acta Biomembr., 1373: 47-58.
28. Salih, A.M.M., S.M. El-Sanousi and I.E.M. El-Zubeir, 2011. A review on the Sudanese traditional dairy products and technology. Int. J. Dairy Sci., 6: 227-245.
29. Abdullah, S.A. and M.M. Osman, 2010. Isolation and identification of lactic acid bacteria from raw cow milk, white cheese and Rob in Sudan. Pak. J. Nutr., 9: 1203-1206.
30. Mustopa, A., 2009. Collection protocol for molecular virology laboratory. Biotechnology Research Center, LIPI Indonesia, Bogor.
31. Bol'shakova, A.V., I.A. Golutvin, N.S. Nasikan and I.V. Yaminskii, 2004. Determination of mechanical characteristics of surface of block copolymers by atomic force microscopy techniques. Polym. Sci. Ser. A, 46: 926-932.
32. Bozzola, J.J. and L.D. Russell, 1999. Electron Microscopy: Principles and Techniques for Biologists. 2nd Edn., Jones and Bartlett Learning, Sudbury, Canada, ISBN: 9780763701925, Pages: 670.
33. Ndaw, A.D., M. Faid, A. Bouseta and A. Zinedine, 2008. Effect of controlled lactic acid bacteria fermentation on the microbiological and chemical quality of moroccan sardines (*Sardina pilchardus*). Int. J. Agric. Biol., 10: 21-27.
34. Lawalata, H.J., L. Sembiring and E.S. Rahayu, 2010. Lactic acid bacteria on bakasang and inhibitory activity against bacterial pathogens and spoilage. Proceedings of the National Seminar of Biology at the Faculty of Biology, September 24-25, 2010, Yogyakarta.
35. Oscariz, J.C. and A.G. Pisabarro, 2000. Characterization and mechanism of action of cerein 7, a bacteriocin produced by *Bacillus cereus* Bc7. J. Applied Microbiol., 89: 361-369.
36. Nes, I.F. and H. Holo, 2000. Class II antimicrobial peptides from lactic acid bacteria. Biopolymers, 55: 50-61.
37. Bizani, D. and A. Brandelli, 2002. Characterization of a bacteriocin produced by a newly isolated *Bacillus* sp. strain 8A. J. Applied Microbiol., 93: 512-519.
38. Brammavidhya, S. and G. Usharani, 2013. Isolation and purification of low molecular weight peptide from marine *B. cereus* and its antimicrobial activity. Int. J. Res. Mar. Sci., 2: 1-5.
39. Ocana, V.S., A.A.P. de Ruiz Holgado and M.E. Nader-Macias, 1999. Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain Applied Environ. Microbiol., 65: 5631-5635.
40. Dalmau, M., E. Maier, N. Mulet, M. Vinas and R. Benz, 2002. Bacterial membrane injuries induced by lactacin F and nisin. Int. Microbiol., 5: 73-80.

41. Brotz, H., G. Bierbaum, K. Leopold, P.E. Reynolds and H.G. Sahl, 1998. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. Antimicrob. Agents Chemother., 42: 154-160.
42. Oktaviani, D., 2004. The effectiveness of bacteriocins of *Lactobacillus plantarum* to the shelf life of red tilapia fillet at low temperatures. Faculty of Fisheries and Marine Science, University of Padjadjaran, Indonesia.
43. Rostini, I., 2007. The role of lactic acid bacteria (*Lactobacillus plantarum*) on the shelf life of red tilapia fillet at low temperatures. Faculty of Fisheries and Marine Science, University of Padjadjaran, Indonesia.
44. Saputra, D. and T. Nurhayati, 2014. Preservation techniques of red tilapia fish fillet with antibacterial compound origin of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Comtech. J. Indonesia, 5: 1021-1030.
45. Udhayashree, N., D. Senbagam, B. Senthilkumar, K. Nithya and R. Gurusamy, 2012. Production of bacteriocin and their application in food products. Asian Pac. J. Trop. Biomed., 2: S406-S410.
46. Atika, L.N., 2014. *Lactobacillus plantarum* as biopreservative on snapper fillet (*skinless*) during chilling temperature storage. Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural Institute, Bogor.