In Vitro Antibacterial Effect of *Lactobacillus plantarum* Postbiotics Against Fish Bacterial Pathogens

Wai Yan Shin[1], Chun Yao Ang[2], Leong Seng Lim[3], Annita Seok Kian Yong[4], Sujjat Al Azad[5], Motohiko Sano[6], Nor Azman Kasan[7], Ibnu Bangkit Bioshina Suryadi[8] And Mohammad Tamrin Mohamad Lal[9*]

[1,2,3,4,5,9*]Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[6]Laboratory of Fish Pathology, Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan
[7]Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Kuala Nerus, 21030, Terengganu, Malaysia
[8]Department of Fisheries, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, West Java, Indonesia

***Corresponding Author:** Mohammad Tamrin Mohamad Lal
*****Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
Tel: 088-320121, Fax: 088-320261 *E-mail Address: mdtamrin@ums.edu.my

Abstract
The intensification of aquaculture farms leads to stressful conditions for fish. This causes the outbreak of bacterial diseases and lowers production in aquaculture. Probiotics and chemical treatments are effective, but it possesses a risk to the environment and human health. Postbiotics emerged to become one of the treatments for bacterial diseases. In this study, *Lactobacillus plantarum* GS12 and GS13 strains were used to determine the antibacterial effect of postbiotics on different pathogenic bacteria. The postbiotics were extracted and both strains show positive inhibition in the screening test. The postbiotics from both strains of *L. plantarum* were then used for further testing on minimum inhibitory concentration. Postbiotic from GS12 showed no inhibition activity, whereas GS13 has the lowest inhibition concentration of 8.0 µg ml^{-1} when tested on *Aeromonas hydrohila* and *Vibrio harveyi*, and 16.7 µg ml^{-1} when tested on *A. salmonicida* and *V. parahaemolyticus*. Postbiotic produced by *L. plantarum* GS13 had better capacity in terms of antibacterial effect compared to *L. plantarum* GS12. *L. plantarum* GS13 postbiotics may be useful against bacterial disease in the future. This study shows a potential alternative control measure for bacterial disease often occurring in aquaculture.

Keywords: Probiotic/ Prevention method/ Bacterial disease/ *Vibrio*/ *Aeromonas*

Introduction

Bacterial pathogens are one of the major causes of infectious diseases and mortality in wild fish stocks and fish cultivated in confined conditions. Infectious diseases are the main cause of economic losses in aquaculture (Tavares-Dias and Martins, 2017) by reducing the overall performance of cultured fish. Bacterial infections occur both in freshwater and marine fish. Among them, *Vibrio* spp. and *Aeromonas* spp. were widely reported to cause bacterial infection in marine and freshwater fish species, respectively (Ina-Salwany *et al.*, 2019; Borella *et al.*, 2020). Through the discovery of growth-promoting and disease-fighting capabilities of antibiotics, fish farmers started to incorporate such drugs in animal feeds. With the development and widespread of antibiotics, death from infectious diseases has reduced dramatically. However, this has contributed to the development of antibiotic resistance in the environment (Reverter *et al.*, 2020). When resistance develops, the antibiotic is no longer capable of controlling the disease caused by the infective agent.

The use of probiotics to inhibit the growth of pathogens is viewed as an alternative to antibiotic treatment (Silva *et al.*, 2020). Probiotics have various beneficial effects on fish. They can help to improve fish health and performance and improve digestion in fish (Balcazar *et al.*, 2006). The introduction of probiotics in aquaculture not only can improve the feed value, but they could inhibit the growth of pathogens, increase immune response in fish and act as growth-promoting factors (Vine *et al.*, 2006).

Postbiotic is a newly emerged term. It was described as non-viable bacterial products or metabolic by-products formed from probiotic microorganisms that resemble the probiotics' favourable therapeutic effects. (Patel *et al.*, 2013). The lack of cellular components in postbiotics, which lowers the danger of microbial translocation, infection, or heightened inflammatory responses, is considered to make them safer than probiotics (Taverniti and Guglielmetti, 2011; Aguilar-

Toalá *et al.*, 2018). Postbiotics have also been reported in inhibiting opportunistic pathogens that cause infection in animals (Kareem et al, 2014).
Lactobacillus plantarum is a Gram-positive, short-rod, acid-tolerant and a heterofermentative group of *Lactobacilli* (Arasu *et al.*, 2013). Among all the other lactic acid bacteria, *L. plantarum* is the most versatile species with useful properties and is commonly found in fermented food products (Guidone *et al.*, 2014). It has several properties which can prevent the growth of bacteria, which includes probiotic properties, antimicrobial activity, antifungal effects, antioxidant properties, and antimutagenic activity (Behera *et al.*, 2018).

The antibacterial activity of *L. plantarum* is commonly studied against various pathogens. *L. plantarum* inhibited *Vibrio parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. harveyi* and *V. anguillarum* (Koga *et al.*, 1998; Nguyen Thi Truc *et al.*, 2019). The previous studies reported that *L. plantarum* GS12 and GS13 showed the antagonistic effects on the pathogen (Ang and Lal, 2019) and the *L. plantarum* GS12 postbiotic possessed an antifungal effect on marine oomycete, *Lagenidium thermophilum* (Joning *et al.*, 2021). This study was then conducted to investigate the antibacterial potential of *L. plantarum* GS12 and GS13 postbiotics. This study may contribute to add-on value to the postbiotic of *L. plantarum* which potentially has a wide antimicrobial spectrum against aquaculture pathogens.

Materials and Methods

Bacterial Samples and Pathogens

Two strains of *L. plantarum*, *L. plantarum* GS12 and GS13, used in this study were isolated from the gut of white leg shrimp, *Litopenaeus vannamei* (Ang and Lal, 2019). The bacterial stocks were readily available in Borneo Marine Research Institute bacterial culture collection. In order to represent freshwater and marine bacterial pathogens, respectively, two species from the genus *Aeromonas* and two species from the genus *Vibrio* were utilised in this study. The selected *Aeromonas* species was *Aeromonas hydrophila* ATCC7965 and *A. salmonicida* ATCC33658. Meanwhile, *V. harveyi* ATCC35084 and *V. parahaemolyticus* ATCC17802 were selected for *Vibrio* species. All four pathogens used for this study were obtained from the American Type Culture Collection. The bacteria were cultured and maintained in TSB media for 24 hours before tested. For marine bacterial pathogens, the TSB media was supplemented with 2% NaCl.

Postbiotics Extraction

Solvent extraction method was used for extraction of postbiotic from both strains of *L. plantarum*. The method was modified based on the study of Lv *et al.* (2017). Approximately 80µl of *L. plantarum* was inoculated into MRS broth and incubated at room temperature for 24 hours. About 15ml of *L. plantarum* culture was poured into a conical centrifuge tube (FALCON) and centrifuged at 5000rpm for 15 minutes. The supernatant was collected and the pH of the supernatant was adjusted to pH7 using sodium hydroxide (NaOH). Ethyl acetate was added into the pH-adjusted supernatant at 1:1 ratio and mixed thoroughly. The solution was leave for approximately 30 minutes at room temperature to form layers (organic and aqueous layers). Organic layer was collected and the solvent was evaporated using a rotary evaporator. Tris buffer pH 7.0 was added at one fifth of original volume of bacterial supernatants. The extract was mixed and stored 4 °C before used.

Postbiotic Concentrations

Currently, there is no standardized method reported to evaluate the concentration of postbiotics. Therefore, this study used Bradford protein assay for determination of the postbiotic concentration (Kielkopf *et al.*, 2020). This study used Biobasic Bradford Reagent and the protocol followed its manufacturer's instructions. The protein standard curved was prepared using bovine serum albumin (BSA). BSA stock solution was prepared into six standard solutions of concentration 5, 20, 40, 60, 80 and 100 µg ml^{-1}. About 30 µl of standard solutions and *L. plantarum* postbiotic extracts were transferred to empty test tubes. Each test tube received 1.5 ml of Bradford reagent, which was thoroughly mixed in. The mixes were incubated for five minutes at room temperature. With the aid of a UV/Vis spectrophotometer, the absorbance is measured at 595 nm. The protein concentration of both postbiotic was determined using the formula derived from the standard curved.

Antibacterial Effect of Postbiotics

Agar well diffusion method (Chen *et al.*, 2019) was used to determine the antimicrobial effect of postbiotic from *L. plantarum*. A hole was made in each of the four parts of the agar plates using a sterile 5mm cock borer. The drilled hole was filled with about 20 l of TSA solution to seal off the bottom. Before use, the agar plates were allowed to completely dry. A sterile cotton bud was used to evenly distribute bacteria pathogens on top of the molten agar. Each postbiotic extract was inoculated into the wells with around 100 l. As a control, sterile Tris buffer pH 7.0 was used. The agar plates underwent a 24-hour incubation period at 25 °C. The antimicrobial effect was determined by observing the presence of inhibition growth zone of bacterial pathogens (Hafidh *et al.*, 2011) after 24 hours of incubation period.

Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) of postbiotic against bacteria pathogens was determined by using modified broth microdilution method by Balouiri *et al.* (2016). The initial concentration of the postbiotic extract was regard as 100%. It was then diluted to 50%, 25%, 12.5%, 6.25% and 3.125% of the original concentration. The lowest

concentration where the isolate was completely inhibited was known as the minimal inhibitory concentration or MIC. The tests were conducted in triplicates. Bacterial growth was monitored through optical density (OD) of bacterial suspensions by using spectrophotometer with wavelength 600nm (OD_{600}). The optical density of the solutions was determined after incubation at room temperature for 24 hours.

Results

The results of protein concentration and antibacterial effect are shown in Table 1. The Bradford assay result showed that the concentration of protein in the *L. plantarum* GS12 and GS13 extracts were 66.7 µg ml^{-1} and 64.1 µg ml^{-1}, respectively. Screening test showed the postbiotics from both *L. plantarum* have antibacterial activities against all bacterial pathogens (*A. hydrophila* ATCC7965, *A. salmonicida* ATCC33658, *Vibrio harveyi* ATCC35084 and *V. parahaemolyticus* ATCC17802). They showed transparent inhibition zone on the media inoculated with aquaculture bacterial pathogens (Figure 1).

Table 1 Protein concentration of postbiotic *L. plantarum* GS12 and GS13 and their antibacterial activities against *V. harveyi*, *V. parahaemolyticus*, *A. salmonicida* and *A. hydrophila*

Postbiotic	**Protein Concentration (µg ml^{-1})**	**Antibacterial Activity***			
		V. harveyi ATCC 35084	*V.parahaemolyticus* ATCC 17802	*A. salmonicida* ATCC 33658	*A. hydrophila* ATCC 7965
L.plantarum GS12	66.7	+	+	+	+
L.plantarum GS13	64.1	+	+	+	+

***** + = Positive; - = Negative

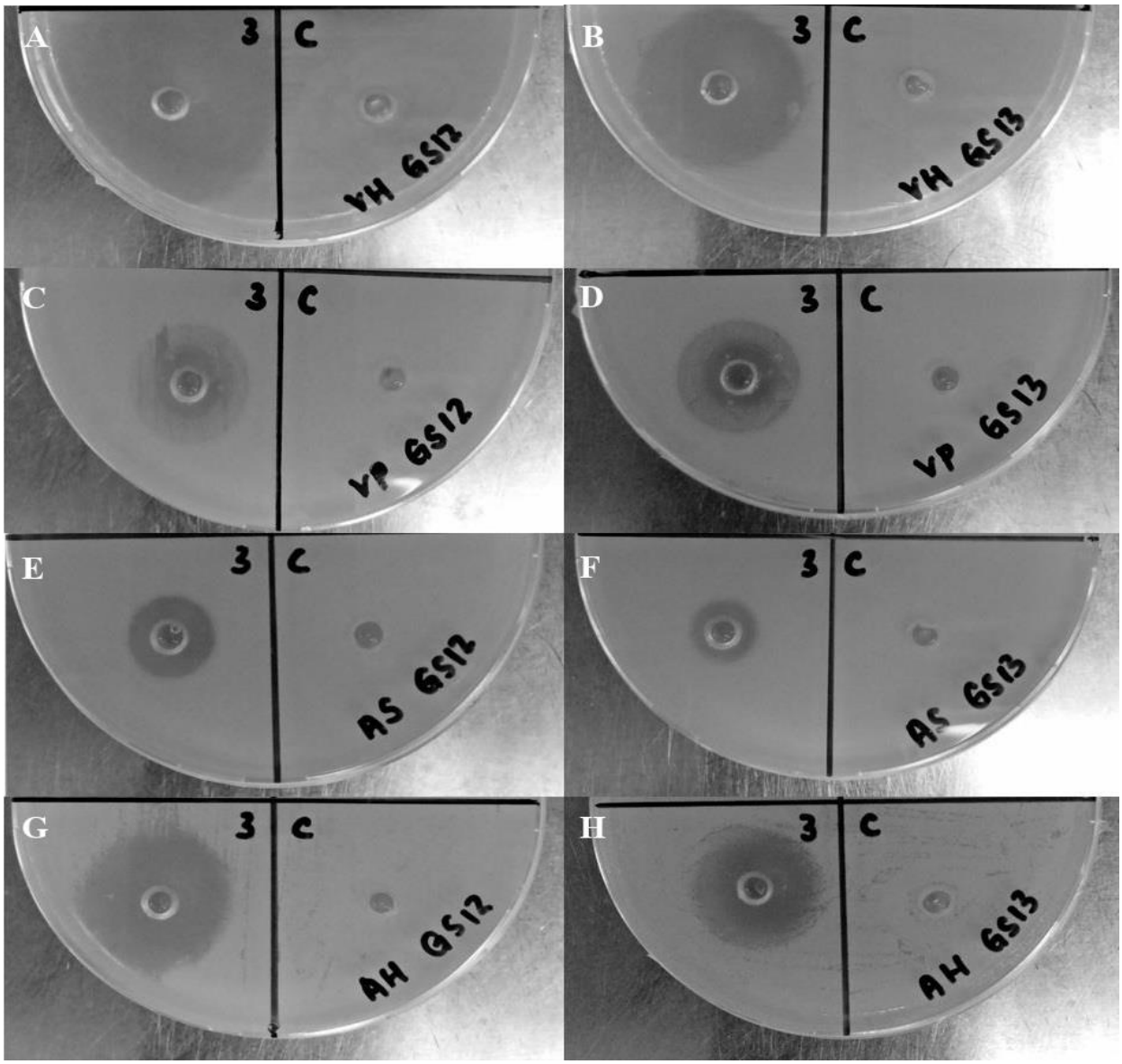

Figure 1: Antibacterial activity of (A) postbiotic extract of *L. plantarum* GS12 against *V. harveyi*, (B) postbiotic extract of *L. plantarum* GS13 against *V. harveyi*, (C) postbiotic extract of *L. plantarum* GS12 against *V. parahaemolyticus*, (D)

postbiotic extract of *L. plantarum* GS13 against *V. parahaemolyticus*, (E) postbiotic extract of *L. plantarum* GS12 against *A. salmonicida*, (F) postbiotic extract of *L. plantarum* GS13 against *A. salmonicida*, (G) postbiotic extract of *L. plantarum* GS12 against *A. hydrophila* and (H) postbiotic extract of *L. plantarum* GS13 against *A. hydrophila.*

As both the postbiotic extracted from GS12 and GS13 showed positive inhibition activity in the antimicrobial screening test, both extracts were carried forward for the minimum inhibitory concentration (MIC) test. Based on the Bradford assay, the concentration (µg ml^{-1}) of *L. plantarum* GS12 and GS13 used in the MIC assay was 33.4, 16.7, 8.3, 4.2, 2.1 µg ml^{-1}, and 32.1, 16.0, 8.0, 4.0, 2.0 µg ml^{-1}, respectively. The result (Figure 2) showed that there was no inhibition activity observed on all aquatic pathogens tested in MIC assay using postbiotic extracted from *L. plantarum* G12. As for *L. plantarum* GS13 postbiotic, the lowest concentration for the postbiotic to inhibit the growth of pathogens was at 8.0 µg ml^{-1}, which was observed on *A. hydrophila* ATCC7965 and *V. harveyi* ATCC35084. Whereas *A. salmonicida* ATCC33658 and *V. parahaemolyticus* ATCC17802 were inhibited at a concentration of 16.7 µg ml^{-1}.

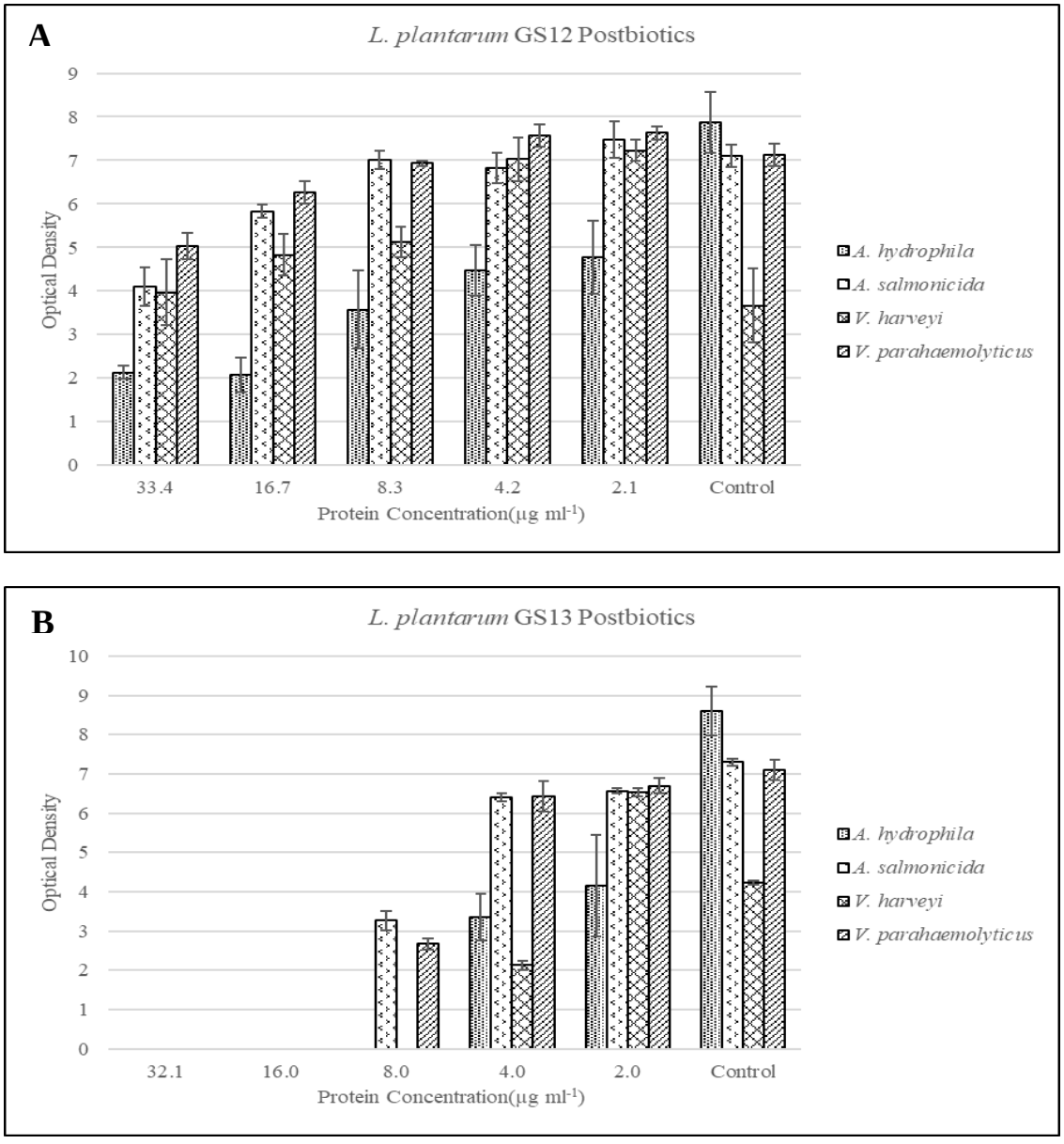

Figure 2: Minimum inhibitory concentration assay of *L. plantarum* GS12 and GS13 to four bacterial pathogens (*A. hydrophila* ATCC 7965, *A. salmonicida* ATCC 33658, *Vibrio harveyi* ATCC 35084 and *V. parahaemolyticus* ATCC 17802) at different concentration of postbiotics. The OD value appear on certain bacterial pathogens indicate that the bacteria growth was not inhibited at that particular concentration.

Discussions

Postbiotic produced by *L. plantarum* GS12 and GS13 strains showed positive antibacterial activities against the four selected pathogens, *A. hydrophila* ATCC7965, *A. salmonicida* ATCC33658, *V. harveyi* ATCC35084, and *V. parahaemolyticus* ATCC17802. The inhibitory effect is portrayed by the clear, distinct zone formed around the wells. The antibacterial activities exhibited by the postbiotic may be explained by the various reports from previous studies.

pH neutralised filtered supernatant of *L. plantarum* inhibited *Pseudomonas fluorescens* (Mahrous *et al*., 2015). Cell-free supernatant (CFS) of *L. plantarum* showed antimicrobial activity against *E. tarda*, *Streptococcus iniae*, *A. veronii*, *P. fluorescens*, *A. hydrophila* (Pimentel and Katagiri, 2008; Kang *et al.*, 2016; Kumar *et al*., 2011; Tremonte *et al*., 2017; Giri *et al.*, 2011). CFS of *L. plantarum* in the study by Todorov *et al*. (2011) and Du *et al.* (2018) inhibited *S. agalactiae* and *P. fluorescens*, respectively, after pH adjustment. pH adjusted and enzyme proteinase K treated CFS of *L. plantarum* also reported able to inhibit *Lac. garvieae* (Pérez-Sánchez *et al.*, 2011). Furthermore, the crude extract of *L. plantarum* obtained with ethyl acetate extraction showed *Aeromonas sobria* biofilm inhibition (Lv *et al.*, 2021).

This study also found that the postbiotics from *L. plantarum* is not species specific. This might be caused by various metabolic by-products such as bacteriocins, peptides, , polysaccharides, enzymes, cell surface proteins, peptidoglycan-derived muropeptides, teichoic acids and organic acids (Aguilar-Toalá *et al.*, 2018) that present inside the postbiotics. A previous study by Lv *et al.* (2018) reported that *L. plantarum* bacteriocin inhibits different species of bacteria. This might also indicate that postbiotics have a wide spectrum of antibacterial effects. A wide spectrum of antibacterial effects may show that postbiotics have almost similar function to antibiotic (Nataraj *et al.*, 2020). The findings in this study also showed that postbiotic produced by *L. plantarum* GS13 had a better capacity to inhibit all the aquatic pathogens used in this study compared to *L. plantarum* GS12. The effect on the pathogens can variate due to the difference in physiological and biochemical properties among different strains of *L. plantarum*. Both *L. plantarum* GS12 and GS13 were reported to differ in term of proteolytic activities (Ang and Lal, 2019).

The bacteria strains used for postbiotic extraction originated from marine shrimp. However, the postbiotics was able to inhibit the growth of freshwater pathogens. This might suggest that probiotic bacteria isolated from various environments had the potential to inhibit different species of bacterial pathogen. Ismail *et al.* (2016) reported that bacteria from macroalgae have antibacterial activities against human pathogens. Thus, this help widens the prospect of the application of marine bacteria to control freshwater bacterial diseases or vice versa. Nonetheless, further research is needed in this aspect.

In conclusion, postbiotics produced from both strains of *L. plantarum* were able to inhibit the activity of pathogens through antibacterial screening tests. Postbiotic extracted from *L. plantarum* GS13 exhibited a higher and obvious inhibitory activity against the four selected pathogens compared to *L. plantarum* GS12. MIC test conducted using postbiotic extracts from GS12 did not show any positive results, as it might require a higher concentration to inhibit the pathogenic activities. Further study should be conducted to determine the exact compound with inhibitory activity of the postbiotics and to optimize the production of the postbiotics. It was also crucial to study on the optimized concentration of postbiotics to be used in aquaculture species so that it might help the aquaculture industry to fight against bacterial disease in the future.

Acknowledgement
Special thanks to the Ministry of Higher Education, Malaysia, for providing the grant to carry out this research under Fundamental Research Grant Scheme (FRGS/1/2019/WAB01/UMS/02/1). This study is also supported by the JSPS Core-to-Core Program “Building up an international research network for successful seed production technology development and dissemination leading South-East Asian region”.

Conflict of Interest
The authors declare no competing interests.

References
1. Aguilar-Toalá, J.E., Garcia-Varela, R., Garcia, H.S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B. and Hernández-Mendova, A., 2018. Postbiotics: An evolving term within the functional food field. Trends in Food Science & Technology, 75, 105-114. https://doi.org/10.1016/j.tifs.2018.03.009
2. Ang, C.Y. and Lal, T.M., 2019. Isolation and characterization of probiotic bacteria from the gastrointestinal tract of pond-cultured *Litopenaeus vannamei* in Tuaran, Sabah. International Journal of Aquatic Science**,** 10(1), 60-73.
3. Arasu, M.V., Al-Dhabi, N.A., Ilavenil, S., Choi, K.C. and Srigopalram, S., 2016. *In vitro* importance of probiotic *Lactobacillus plantarum* related to medical field. Saudi Journal of Biological Sciences, 23(1), S6-S10. https://doi.org/10.1016/j.sjbs.2015.09.022
4. Balcazar, J., Blas, I., Ruizzarzuela, I., Cunningham, D., Vendrell, D. and Muzquiz, J., 2006. The role of probiotics in aquaculture. Veterinary Microbiology, 114(3-4), 173-186. https://doi.org/10.1016/j.vetmic.2006.01.009
5. Balouiri, M., Sadiki, M. and Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005
6. Behera, S.S., Ray, R.C. and Zdolec, N., 2018. *Lactobacillus plantarum* with functional properties: An approach to increase safety and shelf-life of fermented foods. BioMed Research International, 2018, 9361614. https://doi.org/10.1155/2018/9361614
7. Borella, L., Salogni, C., Vitale, N., Scali, F., Moretti, V.M., Pasquali, P. and Alborali, G.L., 2020. Motile aeromonads from farmed and wild freshwater fish in northern Italy: an evaluation of antimicrobial activity and

multidrug resistance during 2013 and 2016. Acta Veterinaria Scandinavica, 62, 6. https://doi.org/10.1186/s13028-020-0504-y

8. Chen, C.C., Lai, C.C., Huang, H.L., Huang, W.Y., Toh, H.S., Weng, T.C., Chuang, Y.C., Lu, Y.C. and Tang, H.J., 2019. Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant *Enterobacteriaceae*. Frontiers in Microbiology, 10, 798. https://doi.org/10.3389/fmicb.2019.00789
9. Du, H., Yang, J., Lu, X., Lu, Z., Bie, X., Zhao, H., Zhang, C. and Lu, F., 2018. Purification, characterization, and mode of action of plantaricin gz1-27, a novel bacteriocin against *Bacillus cereus*. Journal of Agricultural and Food Chemistry, 66(18), 4716-4724. https://doi.org/10.1021/acs.jafc.8b01124
10. Giri, S.S., Sukumaran, V., Sen, S.S., Vinumonia, J., Nazeema Banu, B. and Jena, P.K., 2011. Antagonistic activity of cellular components of potential probiotic bacteria, isolated from the gut of *Labeo rohita*, against *Aeromonas hydrophila*. Probiotics and Antimicrobial Proteins, 3, 214-222. https://doi.org/10.1007/s12602-011-9078-3
11. Guidone, A., Zotta, T., Ross, R.P., Stanton, C., Rea, M.C., Parente, E. and Ricciardi, A., 2014. Functional properties of *Lactobacillus plantarum* strains: A multivariate screening study. LWT - Food Science and Technology, 56(1), 69-76. https://doi.org/10.1016/j.lwt.2013.10.036
12. Hafidh, R.R., Abdulamir, A.S., Vern, L.S., Bakar, F.A., Abas, F., Jahanshiri, F. and Sekawi, Z., 2011. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. Open Microbiology Journal, 5, 96-106. https://doi.org/10.2174/1874285801105010096
13. Ina-Salwany, M.Y., Al-Saari, N., Mohamad, A., Mursidi, F.A., Mohd-Aris, A., Amal, M.N. A., Kasai, H., Mino, S., Sawabe, T. and Zamri-Saad, M., 2019. Vibriosis in fish: A review on disease development and prevention. Journal of Aquatic Animal Health, 31(1), 3-22. https://doi.org/10.1002/aah.10045
14. Ismail, A., Ktari, L., Ahmed, M., Bolhuis, H., Boudabbous, A., Stal, L.J., Cretoiu, M.S., and Bour, M.E., 2016. Antimicrobial activities of bacteria associated with the brown alga *Padina pavonica*. Frontiers in Microbiology, 7, 1702. https://doi.org/10.3389/fmicb.2016.01072
15. Joning, E.J., Yong, A.S.K., Lajimin, S., Ang, C.Y., Ransangan, J., Sano, M., Dan, S., Hamasaki, K., Kasan, N.A. and Lal, T.M., 2021. Antifungal prospect of *Bacillus cereus* postbiotic on crustacean pathogen, *Lagenidium thermophilum*. Biocontrol Science, 26(4), 201-205. https://doi.org/10.4265/bio.26.201
16. Kang, C., Shin, Y., Kim, Y. and So, J., 2016. Isolation of *Lactobacillus* strains from shellfish for their potential use as probiotics. Biotechnology and Bioprocess Engineering, 21, 46-52. https://doi.org/10.1007/s12257-015-0518-x
17. Kareem, K.Y., Ling, F.H., Chwen, L.T., Foong, O.M. and Asmara, S.A., 2014. Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. Gut Pathogens, 6, 23. https://doi.org/10.1186/1757-4749-6-23
18. Kielkopf, C.L., Bauer, W. and Urbatsch, I.L., 2020. Bradford assay for determining protein concentration. Cold Spring Harbor Protocols, 2020(4), 102269. https://doi.org/10.1101/pdb.prot102269
19. Koga, T., Mizobe, T. and Takumi, K., 1998. Antibacterial activity of *Lactobacillus* species against *Vibrio* species. Microbiological Research, 153, 271-275. https://doi.org/10.1016/s0944-5013(98)80011-6
20. Kumar, H., Rangrez, A.Y., Dayananda, K.M., Atre, A.N., Patole, M.S. and Shouche, Y.S., 2011. *Lactobacillus plantarum* (VR1) isolated from an Ayurvedic medicine (Kutajarista) ameliorates *in vitro* cellular damage caused by *Aeromonas veronii*. BMC Microbiology, 11, 152. https://doi.org/10.1186/1471-2180-11-152
21. Lv, X., Cui, T., Du, H., Sun, M., Bai, F., Li, J. and Zhang, D., 2021. *Lactobacillus plantarum* CY 1-1: A novel quorum quenching bacteria and anti-biofilm agent against *Aeromonas sobria*. LWT - Food Science and Technology, 137, 110439. https://doi.org/10.1016/j.lwt.2020.110439
22. Lv, X., Lin, Y., Jie, Y., Sun, M., Zhang, B., Bai, F., Zhao, H. and Li, J., 2018. Purification, characterization and action mechanism of plantaricin DL3, a novel bacteriocin against *Pseudomonas aeruginosa* produced by *Lactobacillus plantarum* DL3 from Chinese Suan-Tsai. European Food Research and Technology, 244, 323-331. https://doi.org/10.1007/s00217-017-2958-3
23. Lv, X., Miao, L., Ma, H., Bai, F., Lin, Y., Sun, M. and Li, J., 2017. Purification, characterization and action mechanism of plantaricin JY22, a novel bacteriocin against *Bacillus cereus* produced by *Lactobacillus plantarum* JY22 from golden carp intestine. Food Science and Biotechnology, 27(3), 695-703. https://doi.org/10.1007%2Fs10068-017-0280-2
24. Mahrous, H., Hussein, H. and El-Bagoury, A.M., 2015. Bio-control of *Pseudomonas fluorescens* in Domiati cheese. Advances in Dairy Research, 3(2), 136. https://doi.org/10.4172/2329-888X.1000136
25. Nataraj, B.H., Ali, S.A., Behare, P.V. and Yadav, H., 2020. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microbial Cell Factories, 19, 168. https://doi.org/10.1186/s12934-020-01426-w
26. Nguyen Thi Truc, L., Trinh Ngoc, A., Tran Thi Hong, T., Nguyen Thanh, T., Huynh Kim, H., Pham Kim, L., Huynh Truong, G., Truong Quoc, P. and Nguyen Thi Ngoc, T., 2019. Selection of lactic acid bacteria (LAB) antagonizing *Vibrio parahaemolyticus*: the pathogen of Acute Hepatopancreatic Necrosis Disease (AHPND) in whiteleg shrimp (*Penaeus vannamei*). *Biology*, 8, 91. https://doi.org/10.3390/biology8040091
27. Patel, R.M. and Denning, P.W., 2013. Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis. Clinics in Perinatology, 40(1), 11-25. https://doi.org/10.1016/j.clp.2012.12.002
28. Pérez-Sánchez, T., Balcázar, J.L., García, Y., Halaihel, N., Vendrell, D., de Blas, I., Merrifield, D. L. and Ruiz-Zarzuela, I., 2011. Identification and characterization of lactic acid bacteria isolated from rainbow trout, *Oncorhynchus mykiss* (Walbaum), with inhibitory activity against *Lactococcus garvieae*. Journal of Fish Diseases, 34(7), 499-507. https://doi.org/10.1111/j.1365-2761.2011.01260.x

29. Pimentel, S.S. and Katagiri, T., 2008. Differences of probiotic effects on *Edwardsiella tarda* challenged Nile tilapia (*Oreochromis niloticus*) fed with four *Lactobacillus* species. Aquaculture Science, 56(3), 401-408.
30. Reverter, M., Sarter, S., Caruso, D., Avarre, J.C., Combe, M., Pepey, E., Pouyaud, L., Vega-Hered ía, S., de Verdal, H. and Gozlan, R.E., 2020. Aquaculture at the crossroads of global warming and antimicrobial resistance. Nature Communications, 11**,** 1870. https://doi.org/10.1038/s41467-020-15735-6
31. Silva, D.R., Sardi, J.C.O., Pitangui, N.S., Roque, S.M., da Silva, A.C.B. and Rosalem, P.L., 2020. Probiotics as an alternative antimicrobial therapy: Current reality and future directions. Journal of Functional Foods, 73, 104080. https://doi.org/10.1016/j.jff.2020.104080
32. Tavares-Dias, M. and Martins, M.L., 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. Journal of Parasitic Diseases, 41(4), 913-918. https://doi.org/10.1007/s12639-017-0938-y
33. Taverniti, V. and Guglielmetti, S. 2011. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: Proposal of paraprobiotic concept). Genes & Nutrition, 6, 261-274. https://doi.org/10.1007/s12263-011-0218-x
34. Todorov, S.D., Prévost, H., Lebois, M., Dousset, X., LeBlanc, J.G. and Franco, B.D.G.M., 2011. Bacteriocinogenic *Lactobacillus plantarum* ST16PA isolated from papaya (*Carica papaya*) - From isolation to application: Characterization of a bacteriocin. Food Research International, 44(5), 1351-1363. https://doi.org/10.1016/j.foodres.2011.01.027
35. Tremonte, P., Pannella, G., Succi, M., Tipaldi, L., Sturchio, M., Coppola, R., Luongo, D. and Sorrentino, E., 2017. Antimicrobial activity of *Lactobacillus plantarum* strains isolated from different environments: a preliminary study. International Food Research Journal, 24(2), 852-859.
36. Vine, N.G., Leukes, W.D. and Kaiser, H., 2006. Probiotics in marine larviculture. FEMS Microbiology Reviews, 30, 404-427. https://doi.org/10.1111/j.1574-6976.2006.00017.x