



DOI: 10.22144/ctu.jen.2021.022

Screening utilization of different natural prebiotic extracts by probiotic *Lactobacillus* sp. for development of synbiotic for aquaculture uses

Huynh Truong Giang*, Vu Hung Hai, Phan Thi Cam Tu, Pham Thi Tuyet Ngan and Vu Ngoc Ut

College of Aquaculture and Fisheries, Can Tho University, Viet Nam

*Correspondence: Huynh Truong Giang (email: htgiang@ctu.edu.vn)

Article info.

Received 22 Feb 2021

Revised 15 Apr 2021

Accepted 04 Jun 2021

Keywords

Lactobacillus, *Litopenaeus vannamei*, natural prebiotic extract, probiotic, synbiotic

ABSTRACT

The study is aimed to develop a relevant synbiotic to promote growth performance of the whiteleg shrimp, *Litopenaeus vannamei*. For this, four common natural fiber extracts from Arcera banana, Siamese banana, yellow sweet potato, and white sweet potato were screened for supporting the growth of *Lactobacillus* sp. which was isolated from whiteleg shrimp intestines with probiotic activity, prebiotic score, and ability to induce bacterial enzyme activities of protease, leu-aminopeptidase, and α -amylase. Results showed that *Lactobacillus* sp. was able to utilize all extracts from banana and sweet potato as the sole carbon sources. At 24 hours of culture, the growth of *Lactobacillus* sp. was highest after adding the extract from white sweet potato as the sole carbon source. Considering pathogenic bacteria, including *Vibrio parahaemolyticus*, white sweet potato extract had the highest prebiotic score with a mean of 0.25 as compared with those of *V. harveyi* with a mean of 0.16. White sweet potato extract induced the highest activities of protease. These results indicated that white sweet potato extract was more suitable for combining with *Lactobacillus* sp. as a synbiotic for shrimp culture.

1. INTRODUCTION

Since 2004, whiteleg shrimp, *Litopenaeus vannamei* has been considered the most popular cultured penaeid species in Asian countries (Liao & Chien, 2011). In Vietnam, shrimp have changed the export turnover of the fishery sector in general and the shrimp export industry in particular. Due to enthusiastic investment and effort, the whiteleg shrimp farming models are becoming superlative and most professional as compared to other species. Advanced and high techniques have been applied to shrimp farming that results in increase of production of whiteleg shrimp, even being able to cope with adverse changes of the farming environment in recent years. It can be agreed that investment for intensification of whiteleg shrimp culture has been of a step toward of aquaculture industry in Vietnam.

A synbiotic is a nutritional supplement which is a combination of probiotics and prebiotics (Cerezuela et al., 2011; Huynh et al., 2017). The synergistic effect of a probiotic is chosen based on its effects on the host, and the prebiotic must specifically stimulate the growth and enzyme activities of the probiotic (Kolida & Gibson, 2011). Among probiotic bacteria, *Lactobacillus* species are potential bacterial strains that can be combined with prebiotic(s) to form the suitable synbiotic products for aquaculture uses, especially for whiteleg shrimp culture (Huynh et al., 2017, 2018a, 2018b). *Lactobacilli* are important microbes that are known for their fermentative ability as well as their health and nutritional advantages and these species also exert antimicrobial activity against pathogens (Rossland et al., 2003). Among prebiotics, isomaltooligosaccharide (IMO)

and galactooligosaccharide (GOS) have been used to combine with *Bacillus megenterium* or *Lactobacillus plantarum* for enhancement of the whiteleg shrimp (Li et al., 2009; Huynh et al., 2018b). In addition, mannan oligosaccharide (MOS), fructooligosaccharide (FOS), or inulin, were also used as prebiotics for synbiotics development. However, Huynh et al. (2017) stated that using these purified commercial prebiotics for developing the synbiotics may increase the feed costs in intensive aquaculture. Therefore, in this study, *Lactobacillus* isolated from whiteleg shrimp's intestine was chosen to screen with several natural prebiotics extracted from natural fruits and vegetables, including Arcera banana, Siamese banana, yellow sweet potato, and white sweet potato which were considered as the sources of natural oligosaccharide (Rosas-Ramírez et al., 2008). The results could provide the evidence for prebiotic extracts utilization by probiotic *Lactobacillus* sp. for further studies in improving growth performance of the whiteleg shrimp culture in the Mekong Delta, Vietnam.

2. MATERIALS AND METHODS

2.1. Chemicals and culture media

De Man, Rogosa and Sharpe (MRS) broth (Difco™ Laboratories, Sparks, MD, USA), tryptone (a pancreatic digest of casein), sodium chloride, dipotassium hydrogen phosphate, Tris-HCl, *p*-nitroaniline (*p*-NA) amino acid, *o*-nitrophenyl-D-galactopyranoside (ONPG), bovine serum albumin (BSA), proteose peptone No.3, beef extract, yeast extract, ammonium citrate, magnesium sulfate, manganese sulfate were used. All chemicals used were analytical grade.

2.2. Probiotic bacteria

Probiotic *Lactobacillus* sp. TV32 strain was isolated from whiteleg shrimp intestine following the standards of *Food and Agriculture Organization/ World Health Organization* (FAO/WHO, 2006) in our previous study (Huynh et al., 2020). The strain was stored at the Laboratory of Beneficial Bacteria for Aquaculture, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University. The *Lactobacillus* sp. was cultured in MRS broth medium combined with skim milk and glycerin at the suitable rate for long-term storage as previously described by Huynh et al. (2018b) and stored at -80°C for further experiments.

2.3. Preparation of prebiotic extracts

This study was carried out from January to December 2019 at the Laboratory of Beneficial Bacteria for

Aquaculture, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University. Crude natural prebiotics were extracted from several common fruits and root vegetables, including Arcera banana variety, Siamese banana variety (*Musa acuminata*), orange-fleshed sweet potato variety (*Ipomoea batatas*), and white sweet potato variety (*I. batatas*).

For bananas, the extraction procedure was based on the method previously described by Boonmee & Rengpipate (2015). Banana was peeled and sliced thinly. Ten grams of sliced bananas were grounded in 100 mL of 100°C water and then stood for 10 mins. The supernatant was filtered and centrifuged at 10,000 rpm at 4°C for 10 mins and then collected the clear solution and freeze-drying until powder form. The powder used as banana extract (BE) was used for the experiment. In order to gain a large volume, the volume of BE was sufficiently extracted for the experiment then refrigerated at 4 °C for later uses.

For sweet potatoes, two sweet potato varieties were used. Sweet potatoes (500 g) were steamed at 100°C for 30 mins. The mixture was dried at 55°C for 18 hours. Next, the mixture was crushed and sifted through the mesh until the mixture was powdered. Then, 10 g of potato starch were dissolved in 100 mL of 70% ethanol, stirred for 15 hours at room temperature. The mixture was filtered through glass filter paper and centrifuged at 10,000 rpm for 10 mins to remove impurities. Then the collected filtrates were subjected to rotary evaporator to remove the ethanol then dried in a vacuum freeze dryer for further use (Marlida et al., 2014). Thus, after extraction, there were four mixtures for evaluation of prebiotic activity in total. The extracts were stored at -4°C for further uses.

2.4. Prebiotic utilization of probiotic *Lactobacillus* sp.

The purpose of the experiment was to evaluate the prebiotic utilization, in which prebiotic extracts was used as the sole of carbon sources for lactic fermentation. The method for evaluation was based on Huynh et al. (2018b). Briefly, the probiotic *Lactobacillus* sp. firstly cultured in MRS medium at 37°C for 24 hours, and then the bacteria were centrifuged at 3000 rpm for 10 mins at 4°C. The bacteria were washed twice and re-suspended in a modified-MRS medium (*m*-MRS). Subsequently, the bacterial solution was inoculated on *m*-MRS agar plates containing 2% of the natural prebiotic extract with Bromocresol purple (BP) indicator. The ability of *Lactobacillus* sp. to utilize extract as a source of carbon was

observed through the discoloration of the surrounding environment from violet to yellow. In addition to the extracts from natural products, *Lactobacillus* sp. that cultured on the m-MRS without extracts/glucose and with glucose were served as negative and positive controls, respectively.

2.5. Evaluation of the growth stimulation of *Lactobacillus* sp. by prebiotic extracts

Lactobacillus sp. TV32 bacteria were prepared as described in Section 2.4. Added 5 mL of *Lactobacillus* sp. to 95 mL of m-MRS medium, and then the bacterial solution was then transferred to 15 mL test tubes. Growth of *Lactobacillus* sp. was assessed as increases in absorption at a wavelength of 600 nm at times 0, 2, 4, 6 and 24 hours using a spectrophotometer (Alpha Helios, Thermo Fisher Scientific, USA). The pH was also monitored through the process as one of the indirect indicators of the growth and digestion of carbohydrate mixtures of the extract. Each culture was run in triplicate (Huynh et al., 2018b).

2.6. Evaluation of the ability of prebiotic extracts to induce growth of *Vibriosis*

Prebiotic extracts were evaluated for the ability of the pathogenic bacteria to stimulate growth. Some common pathogenic strains that are known as the disease causative agents to whiteleg shrimp, including *Vibrio parahaemolyticus* and *V. harveyi* were selected for evaluation. The method was as following a study of Huynh et al. (2018b). Briefly, bacterium *V. harveyi* or *V. parahaemolyticus* was prepared by growing on the medium containing tryptone, soytone, NaCl with 2% glucose or 2% of the natural prebiotic extract. The cultures were then incubated at 28°C with constant agitation and OD₆₀₀ values were recorded at a time interval of 0, 2, 6, and 24 hours. Each culture was run in triplicate.

2.7. Evaluation of prebiotic activity score

The prebiotic score reflects the ability of an oligosaccharide mixture to give probiotics better growth than other bacteria groups (not target bacteria) in the intestinal tract of shrimp and compared with using glucose as a form of sugar needed for the development of lactic bacteria. Positive prebiotic carbohydrates if they are digested well as glucose by beneficial bacteria, and complementary synbiotic are selective by probiotic in digestion while the group of pathogenic bacteria limits digestion. Negative prebiotic score when *Lactobacillus* sp. developed in environments with prebiotic supplements lower than glucose supplementation medium and/or poorer

growth of pathogenic bacteria. The prebiotic score was evaluated according to the method of Mazzola et al. (2015) with slight modifications by Huynh et al. (2018b). *Lactobacillus* sp. cultured in m-MRS medium supplemented with 2% glucose or 2% prebiotic extract as a carbon source. Optical density (OD) values were recorded at 0 and 24 h. The prebiotic score was calculated as previous study of Huynh et al. (2018b)

2.8. Enzyme activity of *Lactobacillus* sp. induced by prebiotic extracts

The ability of *Lactobacillus* sp. in secretion and increases of enzyme activity are important implications for prebiotic utilization and proteolysis that are essential for probiotic biomass growth. Therefore, the purpose of this test was to screen the prebiotic extract(s) that can induce the highest activity in terms of protease derived from *Lactobacillus* sp. The probiotic was cultured in m-MRS medium with 2% of each natural prebiotic extracts and medium containing 2% glucose was served as a control. After 24 hours of incubation at 37°C, the cells were removed by centrifugation at 3000 rpm for 10 mins at 4°C and the cell-free supernatants (CFS) obtained. The protease activity of CFS of *Lactobacillus* sp. were evaluated according to the method described by Huynh et al. (2018b). For this, the protease activity was assayed at 40°C in 100 mmol/L Tris-HCl buffer (pH 9.0). The culture (100 µL) was incubated with 100 µL of a 1% casein solution (prepared in Tris-HCl buffer, pH 7.0) for 10 mins at 37°C. The reaction was stopped by adding 500 µL of 5% (v/v) Trichloroacetic acid. After 20 mins, the contents were centrifuged at 3000 ×g and 4°C for 20 mins, and the supernatant was measured by a modified Lowry's method. One unit of protease was equivalent to the amount of enzyme required to release 1 µg of tyrosine/mL/min under standard assay conditions.

3. RESULTS

3.1. Prebiotic utilization of probiotic *Lactobacillus* sp.

After 24 and 48 hours, the growth of *Lactobacillus* sp. was observed clearly by the colonies in disk containing glucose and natural prebiotic extracts from Arcera banana, Siamese banana, orange-fleshed sweet potato, and white sweet potato as compared to the negative control (-) (Figures 1&2). It is, therefore, concluded that the prebiotic extracts could be used as the sole of carbon sources for lactic fermentation by *Lactobacillus* sp.

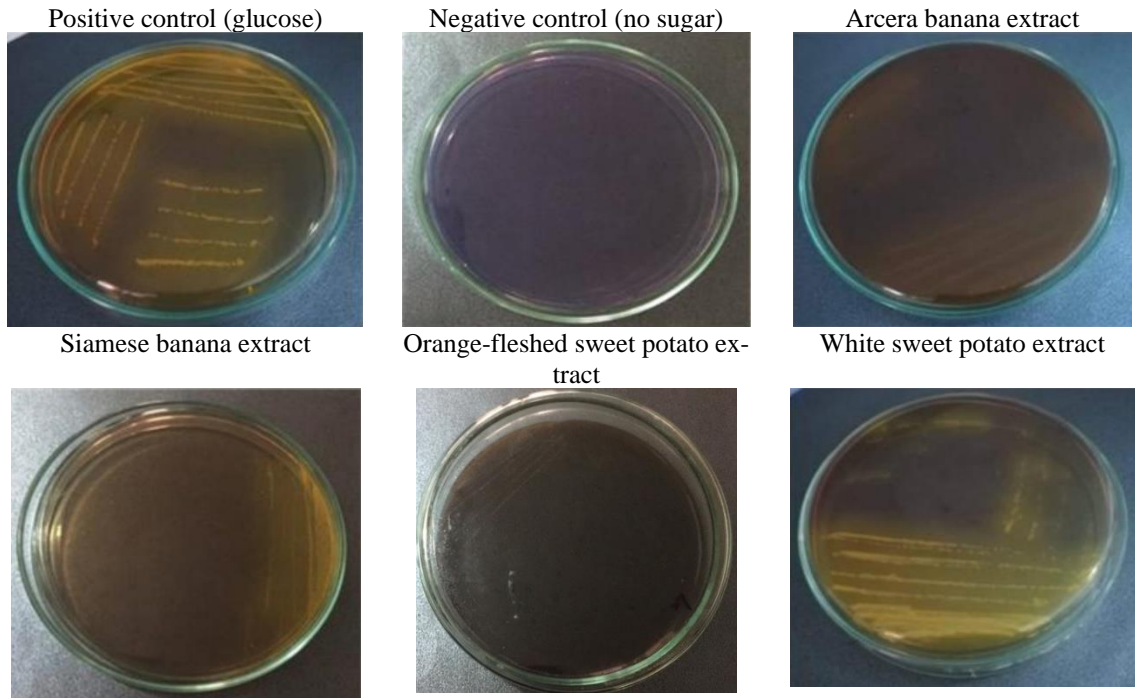


Figure 1. Natural prebiotic extracts utilization ability of the probiotic *Lactobacillus* sp. after 24 hours

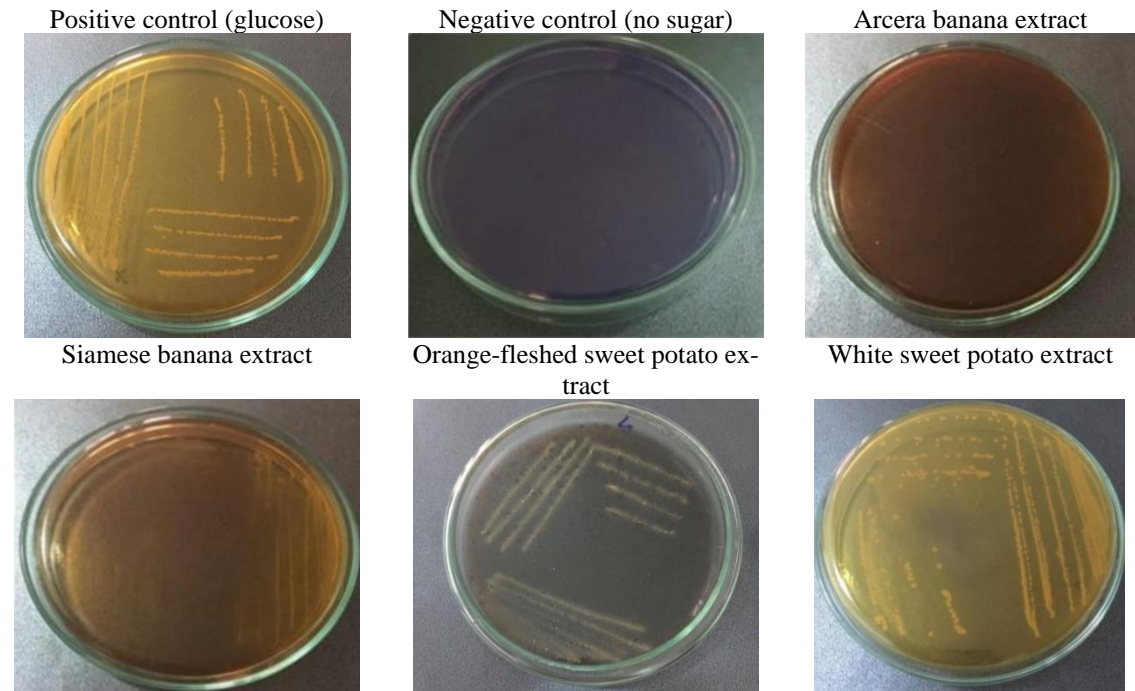


Figure 2. Natural prebiotic extracts utilization ability of the probiotic *Lactobacillus* sp. after 48 hours

3.2. Evaluation of the growth stimulation of *Lactobacillus* sp. by prebiotic extracts

Growth stimulation of *Lactobacillus* sp. by natural prebiotic extracts is shown in Figure 3. Growth levels of *Lactobacillus* sp. among natural prebiotic extracts and glucose did not significantly differ after 6 hours of culture. However, growth of *Lactobacillus* sp. significantly increased in the treatment adding

with natural prebiotic extracts as the sole carbon sources as compared with those of negative control (no sugar added). Results also indicated that the growth of *Lactobacillus* sp. was highest in the medium containing white sweet potato extract as the sole carbon source over 24 hours. Therefore, white sweet potato extract was selected for further tests.

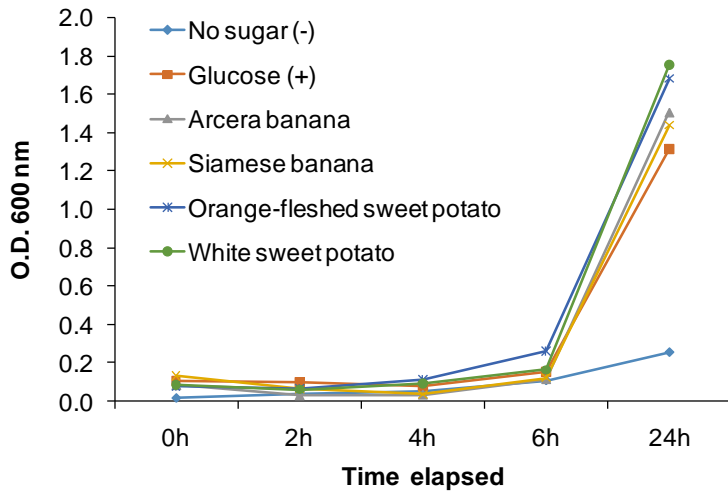


Figure 3. Growth stimulation of *Lactobacillus* sp. by natural prebiotic extracts

No sugar or glucose respectively served as the negative and positive controls

3.3. Evaluation of the ability of prebiotic extracts to induce growth of *Vibriosis* and prebiotic scores

Figures 4 show the white sweet potato extract stimulated the growth of pathogenic bacteria *V. parahaemolyticus*. Obviously, the optical densities of *V. parahaemolyticus* bacterial suspensions at 2 and 24 hours were 0.338 and 1.259, respectively. However, results also were assumed that the growth of *V.*

harveyi did not affected by sugar as the sole of carbon source. The pathogenic bacteria were used as references and averaged scores obtained for the *Lactobacillus* sp. Considering *V. harveyi* and *V. parahaemolyticus*, the mean prebiotic scores were 0.16 and 0.25, respectively (Figure 5). The prebiotic scores indicated that among the *V. harveyi* and *V. parahaemolyticus* community, white sweet potato extract could more stimulate growth of *V. harveyi* than *V. parahaemolyticus*.

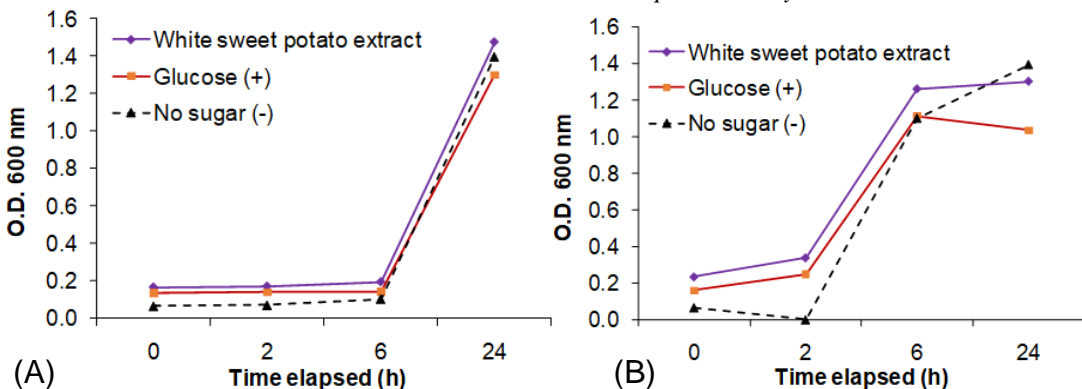


Figure 4. Growth stimulation of *V. parahaemolyticus* (A) and *V. harveyi* (B) by white sweet potato extract

No sugar or glucose respectively served as the negative and positive controls

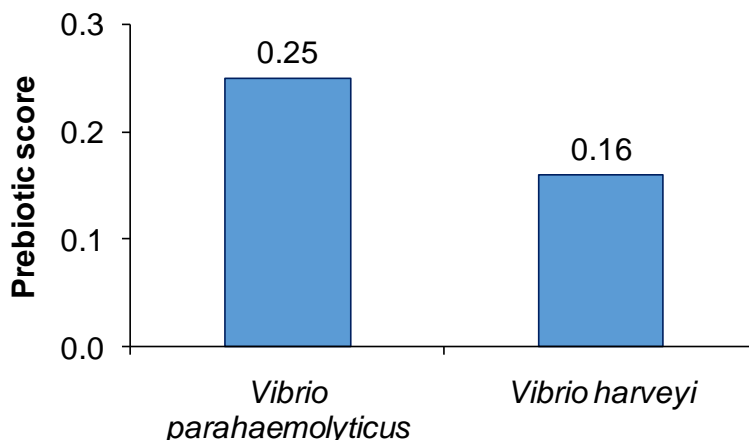


Figure 5. Prebiotic scores of white sweet potato extract obtained from *Lactobacillus* sp. considering and *V. harveyi* and *V. parahaemolyticus*

3.4. Extracellular protease activity of *Lactobacillus* sp. induced by prebiotic extracts

Lactobacillus sp. cultured with white sweet potato extract as the sole carbon source produced

significantly higher protease activity than when cultured with glucose (positive control). Protease enzyme activity was a mean of 208 ± 6.8 units/mL/min (Figure 6).

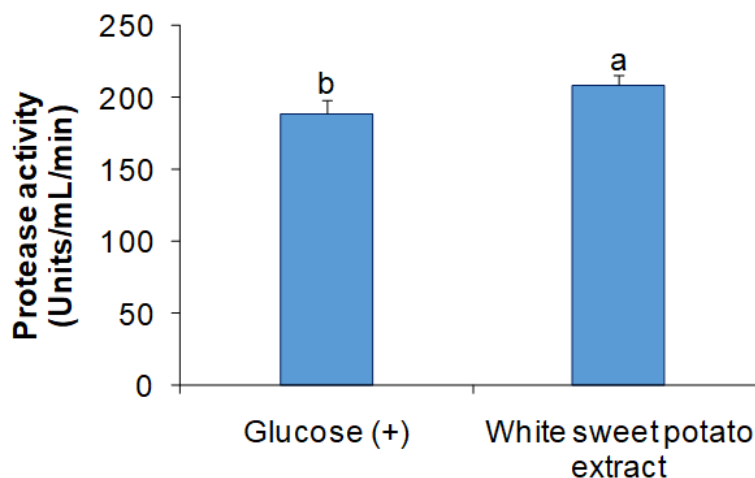


Figure 6. Activities of protease enzymes produced by *Lactobacillus* cultured in the modified MRS medium containing white sweet potato extract as the sole carbon source

Each bar represents the mean value from triplicate determinations with the standard error. Data with different letters significantly differ ($p < 0.05$) among treatments.

4. DISCUSSION

The extracts from banana (*Musa*) and sweet potato (*Ipomoea*) have been used in combination with probiotic in fish and shrimp. In fact, Widanarni and Tanbiyaskur (2015) stated that extract of sweet potato *I. batatas* var. *sukuh* in conjunction with probiotic *Bacillus* sp. NP5 promoted survival rate, growth

performance of Nile tilapia *Oreochromis niloticus* (15-20 g) after 14 days of feeding. In addition, fish were fed the diet containing the extract and *Bacillus* sp. NP5 significantly improved survival after being challenged with *Streptococcus agalactiae* at the dose of 10^4 CFU/fish. Similarly, Marlida et al. (2014) reported that humpback grouper *Cromileptes altivelis* (4.75 ± 0.02 g) fed combination of the

extract from sweet potato *I. batatas* and probiotics including *Sphingomonas paucimobilis*, *Pseudomonas fluorescens* had significantly higher survival, growth performance and feed efficiency than those of the control. The authors also revealed that hematological parameters (as hemoglobin, hematocrit, phagocytic activity) and digestive enzyme (α -amylase) significantly enhanced. Most recently, Putra and Romdhohah (2019) investigated the effects of probiotic (*Bacillus* NP5) and prebiotic (extracted from *I. batatas* var. *sukuh*,) on growth and digestive enzyme activity of dumbo catfish (*Clarias* sp.). The results revealed that a synbiotic (*I. batatas* var. *sukuh* + *Bacillus* NP5) had the growth enhancing effect and feed utilization compared to other treatment (*I. batatas* var. *sukuh* or *Bacillus* NP5 only) on fish. In the whiteleg shrimp *L. vannamei*, extract from sweet potato *Ipomoea* have been also used as a prebiotic for health benefits. Obviously, shrimp fed the sweet potato *Ipomoea* and probiotic SKT-b®*Vibrio alginolyticus* for 30 days significantly increased growth performance and immune response to co-infection with Infectious Myonecrosis Virus (IMNV) and *V. harveyi* at the dose of 10^3 CFU/shrimp (Nurhayati et al., 2015). Most interestingly, a study of Boonmee and Rengpipat (2015) stated that extract from banana *Musa* (ABB group) and *B. subtilis* S11 in combination form did not show effect on growth performance of shrimp after 90 days of feeding. However, the survival rate of shrimp significantly improved when the shrimp were challenged with *V. harveyi* at dose of 10^7 CFU/mL by immersion for 15 days. Previous studies as mentioned above proved the fact that extracts with prebiotic activity from natural fruits and vegetables have been used for improving in implantation of probiotic bacteria along intestinal tract of aquatic animals, thereby exerting the synergistic effects to the host.

Among available prebiotic products, it is not clear which prebiotic carbohydrate is the most suitable substrate for growth of *Lactobacillus* sp. Among *Lactobacillus* species, prebiotics may utilize diverse mechanisms dependent on sugar linkages and the bacterial strain (Huynh et al., 2017). For instance, in *Lb. acidophilus*, fructooligosaccharides (FOSs) are transported by an adenosine triphosphate (ATP)-dependent binding cassette (ABC) transporter and hydrolyzed by intracellular β -fructofuranosidases (β -FFases). In *Lb. plantarum*, FOS is internalized via a sucrose phosphoenol pyruvate-dependent, phosphotransferase system (PTS) transporter, and hydrolysis is catalyzed by a cytoplasmic β -FFase. *Lactobacillus ruminus* can translocate and hydrolyze

FOS and inulin via the major facilitator superfamily (MFS) transporter. On the other hand, galactooligosaccharide (GOS) is internalized via a galactoside-pentose-hexuronide (GPH)-type lactose permease (LacS) transporters. FOS is catalysed by a cytoplasmic β -FFase, whereas GOS is hydrolysed by two cytoplasmic β -galactosidases (*LacA* and *LacLM*) (as cited in Huynh & Liu, 2018). In addition, to utilize mannan oligosaccharide (MOS) as a sole carbon source, *Lactobacillus* species must contain polysaccharide utilization loci (PULs) namely MAN-PUL1, MAN-PUL2, and MAN-PUL3. Probiotic bacteria lacking MAN-PUL2 are unable to grow on MOS. This statement was also demonstrated in a study reported Huynh et al. (2018b). For this, *Lb. plantarum* 7-40 was able to utilize FOS and GOS as the sole carbon sources, but MOS. In the present work, no fermenting occurred in the negative control (no sugar). In contrast, *Lactobacillus* was able to utilize the extracts from banana (*Musa*), sweet potato (*Ipomoea*), and glucose (positive control) as the sole carbon sources and produced acid end products. However, the sugar contents in the extracts from banana and sweet potato in this study are unknown. Therefore, the investigations of the degree of polymerization of the extracts in this study should be unraveled in further studies.

Till now, the sugar contents in banana *Musa* have been reported by several researchers. For example, Poland (1937) reported that the principal sugars of the ripe banana were definitely shown to be sucrose, maltose, fructose, and glucose, whereas maltose is present in very small amounts in commercial yellow banana (*Musa sapientum*). Additionally, according to Vietnam Ministry of Health (VMoH, 2007), total sugar in Siamese banana accounted for 12.2%, whereas galactose and lactose were absent. However, high amounts of fructose (4.85%) and glucose (4.95%), followed by sucrose (2.39%) and maltose (0.1%). For sweet potato *I. batatas*, Lewthwaite et al. (1997) reported that sweet potato contained fructose, glucose, sucrose, and maltose. This study is in accordance with a previous study which concluded that the sum of glucose, fructose, and sucrose accounted for 85-96% and 17-54% of the total soluble sugars identified in the ethanol extracted fractions of sweet potato (Den et al., 1986). In addition, Lai et al. (2013) studied sugar composition of seven sweet potato cultivars. Results revealed that total sugar content in the fresh sweet potatoes varied from 8.41% to 4.5%, in which maltose content was very low (0~0.39%). Because 49.9~92.4% of total sugars were sucrose, sucrose was the major sugar

composition of fresh sweet potatoes. Interestingly, additions to the monosaccharides, oligosaccharides were also detected in the sweet potatoes. Rosas-Ramírez et al. (2008) identified successfully five oligosaccharides containing in sweet potatoes using ^1H nuclear magnetic resonance (NMR)-based metabolomic analysis. According to research on the sugar compositions in banana and sweet potato above, it could explain why *Lactobacillus* sp. can utilize all extracts from banana and sweet potato as the sole of carbon sources for growth of *Lactobacillus* sp. However, the effects of the extracts in stimulating growth of *Vibrio* was not clear, especially in *V. harveyi* based on the OD₆₀₀ at 24 hours and low prebiotic score (Figures 4B&5) in this study.

Previous studies showed that prebiotic extracted from sweet potatoes could effectively support the growth of probiotic bacteria (Putra et al., 2010). Ringo et al. (2010) stated that prebiotic can selectively support the growth of species of bacteria in the digestive tract of shrimp. Therefore, the development of synbiotic by natural prebiotic extracts, including banana and sweet potatoes may be better in cost production in shrimp culture. To assess the specific stimulation of a prebiotic to a probiotic, measurement of the growth of probiotics that cultured in the medium containing prebiotic as the sole of carbon source likely lacks discipline, because interactions between a selective prebiotic and indigenous microbiota remain unknown (Kolida & Gibson, 2011). Therefore, the effectiveness of a prebiotic depends on its ability to be selectively fermented by and to support the growth of specifically targeted probiotic. Regrettably, this issue has received less attention, although numerous commercial prebiotic and synbiotic products have intensively been used in aquaculture (Huynh et al., 2017). In this study, using *V. harveyi* and *V. parahaemolyticus* as references, the higher prebiotic score indicated that white sweet potato was a suitable prebiotic substrate for *Lactobacillus* sp. instead of using glucose as a carbon source for lactic fermentation.

The genus *Lactobacillus* is a diverse group of microorganisms. The genus comprises over 25 species and the first level of differentiation is based on end-product composition. *Lactobacillus* is an important genus in the fermentation of various plant products, fermented fish products and marine environments. *Lactobacillus* isolates are capable of producing protease. *L. plantarum* strains are able to ferment oligosaccharides which may be used in synbiotic products. Among bacterial enzymes, protease is an enzyme that is able to hydrolyze peptide bonds of

proteins. Sulthoniyah et al. (2015) revealed that protease enzyme from *L. plantarum* isolated from shrimp paste had optimum activity at pH 7.0 with specific enzyme activity of 0.34 U/mg protein (Huynh & Liu, 2018). It is also known that lactic acid bacteria possess a complex proteolytic system, although they are less proteolytic than other microorganisms. In this work, in presence of white sweet potato extract, *Lactobacillus* sp. exhibited higher production levels of protease enzymes that play important roles in protein digestibility as compared to those in the glucose control. The obtained results indicate that the extract from white sweet potato can be more suitable substrate for *Lactobacillus* sp. in this study. However, the effects of dietary combination of *Lactobacillus* sp. and white sweet potato in the whiteleg shrimp *L. vannamei* still need in further research.

5. CONCLUSION

Probiotic *Lactobacillus* sp. can utilize extracts from Arcera banana, Siamese bananas, orange-fleshed sweet potato, and white sweet potato. Extract from white sweet potato induced the highest growth of probiotic *Lactobacillus* sp., higher prebiotic activity; stimulated extracellular protease enzyme activities as compared to the positive control. It is, therefore, recommended that white sweet potato extract was more suitable substrate to combine with *Lactobacillus* sp. to be a relevant synbiotic for aquaculture uses.

ACKNOWLEDGMENTS

This study is funded in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan. We appreciate Ms. Lam Anh Thu and Mr. Huynh Trung Hieu for their assistances with the experiments.

REFERENCES

- Boonmee, R., & Rengpipat, S. (2015). Effects of *Musa* (ABB group) prebiotic and *Bacillus subtilis* S11 probiotic on growth and disease resistance of cultivated Pacific white shrimp (*Litopenaeus vannamei*). *Maejo International Journal of Science and Technology*, 9(3), 370-381. <https://doi.org/10.14456/mijst.2015.28>
- Cerezuela, R., Meseguer, J., & Esteban, M. A. (2011). Current knowledge in synbiotic use for fish aquaculture: A review. *Journal of Aquaculture Research and Development*, 51, 008. <https://doi.org/10.4172/2155-9546.S1-008>
- Van Den, T., Biermann, C.J., & Marlett, J. A. (1986). Simple sugars, oligosaccharides, and starch concentrations in raw and cooked sweet potato. *Journal of*

- Agricultural and Food Chemistry*, 34, 421-425.
<https://doi.org/10.1021/jf00069a010>
- Food and Agriculture Organization/World Health Organization [FAO/WHO] (2006). *Probiotics in food. Health and nutritional properties and guidelines for evaluation. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*.
<http://www.fao.org/3/a-a0512e.pdf>
- Huynh, T. G., Shiu, Y. L., Nguyen, T. P., Truong, Q. P., Chen, J. C., & Liu, C. H. (2017). Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish and Shellfish Immunology*, 64, 367-382. <https://doi.org/10.1016/j.fsi.2017.03.035>
- Huynh, T. G., & Liu, C. H. (2018). *Study on action mechanisms of synbiotic as growth-promoting factor in white shrimp Litopenaeus vannamei* (Doctoral thesis on Aquaculture). National Pingtung University of Science and Technology.
- Huynh, T. G., Cheng, A. C., Chi, C. C., Chiu, K. H., & Liu, C. H. (2018a). A synbiotic improves the immunity of white shrimp, *Litopenaeus vannamei*: Metabolic analysis reveal compelling evidence. *Fish and Shellfish Immunology*, 79, 284-293.
<https://doi.org/10.1016/j.fsi.2018.05.031>
- Huynh, T. G., Shiu, Y. L., Nguyen, T. P., Hien, T. T. T., & Liu, C. H. (2018b). Effects of synbiotic containing *Lactobacillus plantarum* 7-40 and galactooligosaccharide on the growth performance of white shrimp, *Litopenaeus vannamei*. *Aquaculture Research*, 49, 2416-2428. <https://doi.org/10.1111/are.13701>
- Huynh, T. G., Nguyen, H. N. U., Vu, H. H., Pham, T. T. N., & Vu, N. U. (2020). Characterization of potential probiotic *Lactobacillus* from whiteleg shrimp intestines for possible additives in pellet feeding. *Can Tho University Journal of Science. Special Issues on Aquaculture*, (1), 102-111.
<https://doi.org/10.22144/ctu.jsi.2020.012> (In Vietnamese with English abstract).
- Kolida, S., & Gibson, G. R. (2011). Synbiotics in health and disease. *Annual Review of Food Science and Technology*, 2, 373-393. <https://doi.org/10.1146/annurev-food-022510-133739>
- Lai, Y. C., Huang, C. L., Chan, C. F., Lien, C. Y., & Liao, W. C. (2013). Studies of sugar composition and starch morphology of baked sweet potatoes (*Ipomoea batatas* (L.) Lam). *Journal of Food Science and Technology*, 50, 1193-1199.
<https://doi.org/10.1007/s13197-011-0453-6>
- Lewthwaite, S. L., Sutton, K. H., & Triggs, C. M. (1997). Free sugar composition of sweetpotato cultivars after storage. *New Zealand Journal of Crop and Horticultural Science*, 25(1), 33-41.
<https://doi.org/10.1080/01140671.1997.9513984>
- Li, J., Tan, B., & Mai, K. (2009). Dietary probiotic *Bacillus* OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). *Aquaculture*, 291, 35-40.
<https://doi.org/10.1016/j.aquaculture.2009.03.005>
- Liao, I. C. & Chien, Y. H. (2011). The Pacific white Shrimp, *Litopenaeus vannamei*, in Asia: The world's most widely cultured alien crustacean. In B. S. Galil, P. F. Clark, & J.T. Carlton (Eds.), *In the wrong place - Alien marine crustaceans: Distribution, biology and impacts* (p. 716). *Invading Nature - Springer Series in Invasion Ecology* 6. Springer: Dordrecht, the Netherlands. https://doi.org/10.1007/978-94-007-0591-3_17
- Marlida, R., Suprayudi, M. A., Widanarni, & Harris, E. (2014). Growth, digestive enzyme activity and health status of humpback grouper (*Cromileptes altivelis*) fed with synbiotic. *Pakistan Journal of Nutrition*, 13 (6), 319-326. <https://doi.org/10.3923/pjn.2014.319.326>
- Mazzola, E., Perrone, G., & Kamuriwo, D. S. (2015). Network embeddedness and new product development in the biopharmaceutical industry: the moderating role of open innovation flow. *International Journal of Production Economic*, 160, 106-119.
<https://doi.org/10.1016/j.ijpe.2014.10.002>
- Nurhayati, D., Widanarni, & Yuhana, M. (2015). Dietary synbiotic influence on the growth performance and immune response to co-infection with infectious Myonecrosis virus and *Vibrio harveyi* in *Litopenaeus vannamei*. *Journal of Fisheries and Aquatic Science*, 10, 13-23. <https://doi.org/10.3923/jfas.2015.13.23>
- Putra, A. N., & Romdhonah, Y. (2019). Effects of dietary *Bacillus* NP5 and sweet potato extract on growth and digestive enzyme activity of dumbo catfish *Clarias* sp. *Jurnal Akuakultur Indonesia*, 18(1), 80-88.
<https://doi.org/10.19027/jai.18.1.80-88>
- Putra, A. N., 2010. *The study of probiotics, prebiotics and synbiotic to Improve growth performance on Tilapia (Oreochromis niloticus)* (master's thesis). Bogor Agricultural University, Indonesia.
- Ringo, E., Olsen, R. E., Gifstad, T. O., Dalmo, R. A., Amund, H., Hemre, G. I., & Bakke, A. M. (2010). Prebiotics in aquaculture: a review. *Aquaculture and Nutrition*, 16, 117-136. <https://doi.org/10.1111/j.1365-2095.2009.00731.x>
- Rosas-Ramírez, D., Escalante-Sánchez, E., & Pereda-Miranda, R. (2008). Five novel oligosaccharides from sweet potato (*Ipomoea batatas* (L.) Lam). *Planta Medicina*, 74 (9), PH34.
<https://doi.org/10.1055/s-0028-1084879>
- Rosslund, E., Borge, G. I. A., Langsrud, T., & Sorhaug, T. (2003). Inhibition of *Bacillus cereus* by strains of *Lactobacillus* and *Lactococcus* in milk. *International Journal of Food Microbiology*, 89, 205-212.
[https://doi.org/10.1016/S0168-1605\(03\)00149-1](https://doi.org/10.1016/S0168-1605(03)00149-1)
- Sulthoniyah, S. T. M., Hardoko, & Nursyam, H. (2015). Characterization of extracellular protease lactic acid

bacteria from shrimp paste. *Journal of Life Science and Biomedicine*, 5(1), 1-5. <http://jlsb.science-line.com/attachments/article/33/J>.

Vietnam Ministry of Health [VMoH] (2007). *Vietnam food composition table*. Medical Publishing House. In Vietnamese.

Widanarni & Tanbiyaskur (2015). Application of probiotic, prebiotic and pynbiotic for the control of *Streptococcus* in tilapia *Oreochromis niloticus*. *Pakistan Journal of Biological Sciences*, 18, 59-66. <https://doi.org/10.3923/pjbs.2015.59.66>.