ผลของอาหารเสริมโพรไบโอติก แลคโตบาซิลลัส แพลนทารัม T13 ต่อความสามารถในการเติบโตและกิจกรรมของเอนไซม์ต่อกุ้งขาว

Effect of Dietary Probiotics *Lactobacillus plantarum* T13 on Growth Performance and Digestive Enzymes Activity of Pacific White Shrimp (*Litopenaeus vannamei*)

สิรวิชญ์ สร้อยแก้ว[1], หริศ หริรัตนกุล[1], โชคชัย เหลืองธุวปราณีต[2] และ มนทิรา ลีลาเกรียงศักดิ์[3*]

Sirawit Soikaew[1], Haris Harirattanakul[1], Chokchai Luangtuvapraneet[2] and Montira Leelakriangsak[3*]

[1] *โรงเรียนสาธิตมหาวิทยาลัยสงขลานครินทร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี*

[2] *ภาควิชาเทคโนโลยีและการอุตสาหกรรม คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี*

[3] *ภาควิชาวิทยาศาสตร์ คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี*

[1]*Demonstration School of Prince of Songkla University, Prince of Songkla University Pattani campus*

[2]*Technology and Industry Department, Faculty of Science and Technology, Prince of Songkla University Pattani campus*

[3]*Science Department, Faculty of Science and Technology, Prince of Songkla University Pattani campus*

Received : 31 July 2019

Revised : 23 October 2019

Accepted : 15 December 2019

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของโพรไบโอติก *Lactobacillus plantarum* T13 ต่อความสามารถในการเติบโตและกิจกรรมของเอนไซม์ย่อยอาหารของกุ้งขาว โดยใช้อาหารกุ้งทางการค้าทั่วไปเสริมโพรไบโอติก 2 ความเข้มข้น ได้แก่ 10^9 CFU g^{-1} (A) และ 10^5 CFU g^{-1} (B) อาหารกุ้งเสริมโพรไบโอติกถูกเก็บรักษาที่อุณหภูมิ 4 องศาเซลเซียส และเตรียมใหม่ทุก ๆ สัปดาห์ ปรับสภาพกุ้งขาวระยะ P15 เป็นเวลา 10 วัน น้ำหนักเริ่มต้นเฉลี่ย 0.015 ± 0.0016 กรัม โดยแบ่งเป็น 3 ชุดการทดลอง คือชุดควบคุม (C) และอีก 2 ชุดการทดลองคือกุ้งที่เลี้ยงด้วยอาหารเสริมโพรไบโอติก (A และ B) ทดลอง 3 ซ้ำ เป็นเวลา 8 สัปดาห์ โดยตรวจและจัดการคุณภาพน้ำทุกสัปดาห์ เพื่อให้อยู่ในเกณฑ์ที่ยอมรับของการเพาะเลี้ยง เมื่อเปรียบเทียบกุ้งที่ได้รับอาหารเสริมโพรไบโอติก กับชุดควบคุม พบว่าน้ำหนักสุดท้าย น้ำหนักที่เพิ่มขึ้น และกิจกรรมของเอนไซม์โปรตีเอส แตกต่างอย่างมีนัยสำคัญทางสถิติ (P<0.05) สำหรับชุดทดลอง A มีอัตราแลกเนื้อ (2.03±0.15) และอัตราการเติบโตจำเพาะ (9.48±0.14) แตกต่างอย่างมีนัยสำคัญทางสถิติ (P<0.05) เมื่อเทียบกับชุดควบคุม แต่ไม่แตกต่างจากชุดทดลอง B อย่างไรก็ตาม อัตราการรอดชีวิต และกิจกรรมของเอนไซม์อะไมเลส ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (P>0.05) ในการทดลองทั้ง 3 ชุด การศึกษานี้แสดงให้เห็นว่าโพรไบโอติก *L. plantarum* T13 ที่ความเข้มข้น 10^9 CFU g^{-1} สามารถเพิ่มความสามารถในการเติบโตและกิจกรรมของเอนไซม์โปรตีเอสในกุ้งขาวได้

คำสำคัญ : โพรไบโอติก, กุ้งขาว, ความสามารถในการเติบโต, เอนไซม์ย่อยอาหาร

***Corresponding author. E-mail** : montira.l@psu.ac.th

Abstract

This study aimed to investigate the effect of dietary probiotics *Lactobacillus plantarum* T13 on growth performance and digestive enzyme activity of Pacific white shrimp. Two concentrations of T13 were prepared and mixed with commercial shrimp feed to give final concentrations of approximately 10^9 CFU g^{-1} (A) and 10^5 CFU g^{-1} (B). Probiotic shrimp feeds were stored at 4°C and was prepared fresh every week. P15 shrimp, *L. vannamei*, were acclimated for 10 days with the average initial weight of 0.015 ± 0.0016 g and were divided into three groups: control (C) and two treatments fed with probiotic shrimp feed (A and B). The experiment was conducted in triplicate for eight weeks. During the experiment, weekly water quality was monitored and managed to maintain the acceptable aquaculture conditions. In comparison to the control group, final weight, weight gain and protease activity were significantly higher in shrimp fed with probiotics (P<0.05). Significant differences (P<0.05) for feed conversion ratio (2.03±0.15) and specific growth rate (9.48±0.14) in treatment A were observed as compared with the control but not with treatment B. However, no significant differences (P>0.05) were detected for survival rate and amylase activity among all experimental groups. Our study demonstrated that probiotic *L. plantarum* T13 at the concentration of 10^9 CFU g^{-1} could improve growth performance and protease activity of Pacific white shrimp.

Keywords: Probiotic, Pacific white shrimp, growth performance, digestive enzymes

Introduction

Culture and production of the Pacific white shrimp (*Litopenaeus vannamei*) have increased and replaced black tiger shrimp after 2005 in Thailand (Lebel *et al*., 2010). The annual white shrimp production reached to 600,000 tons in 2011(Thitamadee *et al*., 2016). However, disease outbreaks have caused a great loss to the shrimp cultivation industry, particularly from opportunistic and pathogenic bacteria such as *Vibrio* sp. and viruses (Flegel, 2006; Lebel *et al*., 2010). The use of antimicrobial agents for disease control has stimulated the development of multiple antibiotic resistance genes in bacteria (Farzanfar, 2006). Therefore, the use of beneficial bacteria called probiotics to displace pathogenic bacteria by competitive processes in the shrimp digestive system is a better approach than administering antibiotics which provides sustainable aquaculture as well as being environmentally friendly (Desriac *et al*., 2010; Farzanfar, 2006; Gillor *et al*., 2008; Wang *et al*., 2008).

Probiotics are defined as “live microorganisms which when administrated in adequate amounts confer a health benefit on the host” (Pineiro *et al*., 2007). The use of probiotics in shrimp aquaculture has recently received increased attention (Luis-Villasenor *et al*., 2013; Nguyen *et al*., 2018; Wang *et al*., 2019; Zokaeifar *et al*., 2012; Zuo *et al*., 2019). Probiotics provide advantages to their hosts such as inhibitory activity against pathogens, stimulating the immunity, enhancement of digestibility of nutrients, promotion of growth and survival, improvement of water quality and bioremediation (Farzanfar, 2006; Gillor *et al*., 2008; Kewcharoen *et al*., 2019; Martinez Cruz *et al*., 2012;

Nimrat *et al*., 2012; Toledo *et al*., 2019). The inhibitory activity against pathogens result from several compounds that may inhibit the growth of competing bacteria (Farzanfar, 2006). Among these compounds, the bacteriocins are the most important (Desriac *et al*., 2010; Gillor *et al*., 2008).

Lactic acid bacteria and the *Bacillus* genus are among the microorganisms most frequently used as probiotics in marine shrimp culture (Buntin *et al*., 2008; Nguyen *et al*., 2018; Toledo *et al*., 2019; Vieira *et al*., 2013). *Lactobacillus plantarum* T13 is a bacteriocin-producing lactic acid bacterial strain isolated from traditional Vietnamese fermented cabbage (Nguyen *et al*., 2014b). The culture extract from T13 was shown to prolong the chilling preservation of fresh cobia meat (Nguyen *et al*., 2014b) and exerted substantial antimicrobial activities against acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio* strains (Nguyen *et al*., 2018). Besides, the probiotic feed for spiny lobster (*Panulirus ornatus*) prepared by adding three strains including *Bacillus pumilus* B3.10.2B, *Bacillus cereus* D9 and *Lactobacillus plantarum* T13 displayed increased in growth and reduced feed conversion rates (Nguyen *et al*., 2014a). Therefore, the objective of this work was to investigate the efficacy of dietary probiotics *Lactobacillus plantarum* T13 on growth performance and digestive enzymes activity of Pacific white shrimp (*Litopenaeus vannamei*) in Thailand.

Methods

Probiotic preparation

Probiotic *Lactobacillus plantarum* T13 was grown in MRS broth using a shaking incubator at 37°C for 12 h and adjusted OD_{600nm} to 0.05. The culture was then centrifuged at 5000 rpm for 8 min after 6 h of cultivation. The pelleted bacteria were washed 3 times and resuspended in Normal saline solution (0.95%) after discarding the supernatant. The commercial feed (CP 9701, Thailand) was used as the basal diet for the supplementation of T13 in the ratio of feed 1 g: oil 0.05 ml: 0.95% NaCl 0.35 ml to give a final concentration of T13 approximately 10^9 CFU g^{-1} and 10^5 CFU g^{-1}. CP 9701 feed contains protein >35%, fats >5%, crude fiber < 4% and moisture < 11%. The amount of probiotic T13 was examined using a spread plate technique on MRS agar. The probiotic feeds were dried at 37°C for 6 h and kept at 4°C until used. The feeds were prepared fresh every week.

Experimental design

The healthy P15 shrimp, *L. vannamei*, were obtained from a commercial farm (Gong Kanchana 4, Sathing Phra, Songkhla province, Thailand). Shrimp were acclimated for 10 days and fed with regular feed. Five hundred and forty shrimp were evenly distributed in 9 tanks (3 tanks per diet), with tank size of 30 x60 x30 cm, containing 36 l seawater. The control (C) group was fed with un-supplemented diet. The other two groups were fed T13 supplemented diets at two different concentrations 10^9 CFU g^{-1} (A) and 10^5 CFU g^{-1} (B) for 8 weeks. Shrimp were fed four times a day at 7.00, 12.00, 17.00 and 23.00 h and at 3-7% of the body weight.

The water was directly collected from the sea followed by treatment before use. Dissolved oxygen was determined using self-prepared test kit as described by Luangthuvapranit and Srichai, 2007. Temperatures were between 26-31°C, Salinity of 15-25 ppt and pH of 7.0-8.5. Water chemical parameters were measured weekly using PARA TEST kits including ammonia (Aquacare 2000.4, Para Ammonium test range 0.25-10 ppm) and nitrite (SONA Nitrite Test, test range 0.1-3.0 mg l^{-1}). All parameters were measured and recorded within standard ranges, with a 50% water change weekly.

Measurement of shrimp growth and survival

Fifty percent of shrimp in each tank were randomly collected each week to measure weight and length. All survived shrimp were counted weekly. At the end of the experiment, the final weight, survival rate, weight gain (WG), feed conversion ratio (FCR) and specific growth rate (SGR) were calculated according to Zokaeifar *et al*. (2012) with slight modifications.

Weight gain (%) = 100 x (final weight - initial weight) / initial weight

Survival rate (%) = 100 x (final numbers/ initial numbers)

Feed conversion ratio (FCR) = Total feed given (g) / wet weight gain (g)

Specific growth rate (SGR) = 100 x ([ln final wt – ln initial wt]/ days)

Determination of enzyme activity

The crude extract of gastrointestinal tract (GIT) was prepared according to Zokaeifar *et al.* (2012) with modifications. Five individuals from each replicate were randomly collected at the end of week 8 and shrimp GIT of each shrimp was removed aseptically, pooled, weighted and homogenized with cold distilled water at ratio 1:10. The homogenate was centrifuged at 5000 rpm at 4°C for 20 min. The crude extract was separated by filtration through 0.45 µm filters. Aliquots were made in 1.5 ml microcentrifuge tubes in triplicate and kept at -20°C to analyze protease and amylase activity.

Protease activity was calculated from a modified method from Shimogaki *et al.* (1991). The reaction mixture (1 ml) contained 1% casein as substrate and was incubated at 37°C. After incubation for 20 min, the reaction was stopped by the addition of 10% TCA and centrifuged at 7500 rpm for 8 min. The absorbance of supernatant was measured at 280 nm. A standard curve of absorbance at 280 nm was prepared using known concentrations of tyrosine. The total protein concentration was measured using Bio-Rad protein assay as recommended by the manufacturer. Protease activity was defined as the amount of enzyme liberating 1 µg of tyrosine per mg protein per min under the conditions described above.

Amylase activity was determined by dinitrosalicylic acid (DNS) method modified from Dutta *et al.* (2006). 50 µl of crude extract was mixed with 50 µl 1% starch. After 3 min of incubation, 100 µl DNS was added and heated at 100°C for 5 min. Subsequently, the reaction mixture was cooled down until it reached room temperature and the absorbance at 540 nm was measured. The OD values were converted to micrograms of glucose equivalent using

a standard graph obtained from the known concentration of glucose. Enzyme activity was defined as the amount of enzyme that released 1 µg of reducing sugar as glucose standard per mg protein per minute under the assay conditions described above.

Statistical analysis

Data on water parameters, growth parameters and enzyme activity were analyzed by using one-way analysis of variance with F-test and Duncan's multiple range test was used to determine the significant variation (P<0.05) among treatments.

Results

Water quality was measured every week for 8 weeks. Water parameters including DO, temperature, ammonia, nitrite, salinity and pH were determined and recorded within the acceptable ranges of dissolved oxygen of more than 6 mg l^{-1}, the temperature of 26-30°C, ammonia less than 1 mg l^{-1}, nitrite less than 0.1 mg l^{-1}, pH between 7.0-8.5 and salinity between 15-25 ppt as shown in Table 1. The average of water parameters was analyzed and compared each treatment for 8 weeks. The result showed that there was no significant difference among the control and probiotic T13 feed treatments.

Table 1 The average of water quality parameters of all treatments for 8 weeks

Water parameters	Treatments			Mean	Standard
	Control	A	B		
DO (mg l^{-1})	7.1±0.1[a]	7.0±0.1[a]	6.8±0.1[a]	7.0±0.1	>6
Temp (°C)	27.5±0.1[a]	27.4±0.1[a]	27.3±0.1[a]	27.4±0.1	26-31
Ammonia (ppm)	0.63±0.07[a]	0.57±0.06[a]	0.52±0.06[a]	0.57±0.06	<1
Nitrite (mg l^{-1})	0	0	0	0	<0.1
Salinity (ppt)	17.2±0.2[a]	16.8±0.3[a]	16.5±0.3[a]	16.8±0.3	15-25
pH	7.7±0.0[a]	7.7±0.0[a]	7.6±0.1[a]	7.7±0.0	7-8.5

Values (mean±SE) with different superscript in each row show significant differences (F-test, P<0.05)

During the probiotic feed storage, survival rates of probiotic T13 were monitored and results showed that 78-85% survived after 7 days (data not shown). The effect of dietary probiotics T13 10^9 CFU g^{-1} and 10^5 CFU g^{-1}on growth performance including initial weight, final weight, final length, weight gain, FCR, SGR and survival rate was performed for 8 weeks. The analyzed data in different treatments and control are shown in Table 2. Initial weight and survival rate did not differ among treated and control groups. However, the final weight and weight gain of treatment A were recorded and showed a significant difference (P<0.05) compared with treatment B and control groups. Final length, FCR and SGR of treatment A were not significantly different (P>0.05) compared to that of

treatment B but had significantly higher (P<0.05) than those of control. Low survival was observed due to stress and high stock density.

Table 2 Growth performance and survival rate of *L. vannamei* cultured with probiotic T13 and control for 8 weeks

Treatments	Control	A: 10^9 CFU g^{-1}	B: 10^5 CFU g^{-1}
Initial weight (g)	0.015±0.0022^a	0.015±0.0006^a	0.014±0.0020^a
Final weight (g)	1.85±0.41^b	3.09±0.58^a	2.31±0.29^b
Final length (cm)	6.29±0.33^b	7.43±0.26^a	6.90±0.15^{ab}
Weight gain (g)	1.84±0.11^b	3.08±0.32^a	2.29±0.09^b
FCR	2.60±0.18^b	2.03±0.15^a	2.20±0.05^{ab}
SGR	8.46±0.26^b	9.48±0.14^a	9.00±0.21^{ab}
Survival (%)	38.33±6.34^a	33.89±1.47^a	31.67±5.77^a

Values (mean±SE) with different superscript in a row show significant differences (F-test, P<0.05)

The highest protease and amylase activity was recorded for the GIT of shrimp fed with treatment A followed by treatment B and control after 8 weeks of culture as summarized in Figure 1. No significant difference was observed in amylase activity from all treatments. However, protease activity of shrimp fed with T13 10^9 CFU g^{-1} (treatment A) was significantly higher (P<0.05) than those of T13 10^5 CFU g^{-1} (treatment B) and control. This result suggested that the increase of protease activity enhanced the digestion and absorption ability and, thus improved shrimp weight gain.

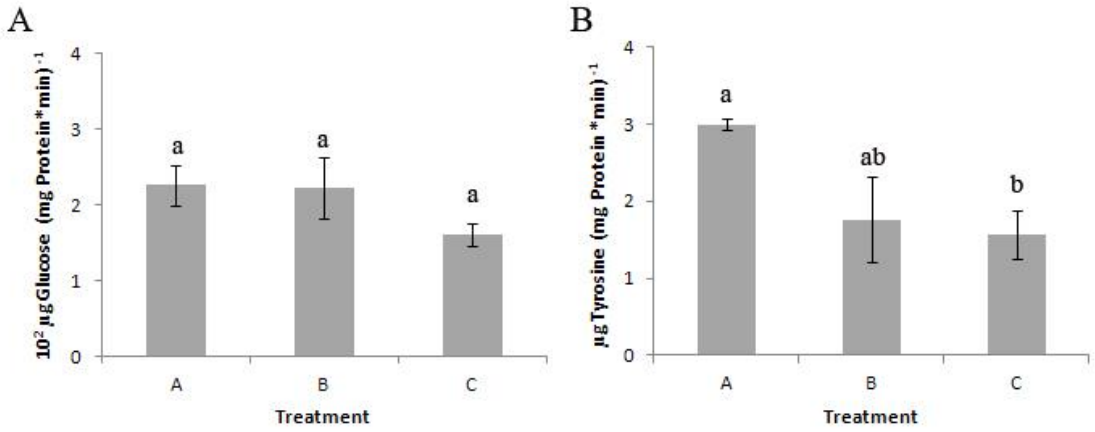

Figure 1 Digestive enzyme activity of *L. vannamei* fed with probiotic T13 at concentrations of 10^9 CFU g^{-1} (treatment A), 10^5 CFU g^{-1} (treatment B) or without probiotic T13 (treatment C) after 8 weeks. A, amylase activity B, protease activity. Values (mean±SE) with different superscript show significant differences (P<0.05).

Discussion

The application of probiotics in aquatic animals is now wildly accepted and is increasing with the demand for environmentally friendly aquaculture (Desriac *et al*., 2010; Dobson *et al*., 2012; Wang *et al*., 2008). The use of probiotics in aquaculture has been demonstrated to have beneficial effect on growth performance, enzymatic contribution to digestion, inhibition of pathogenic microorganisms and increased immune response (Becerra-Dorame *et al*., 2012; Castex *et al*., 2008; Luis-Villasenor *et al*., 2011; Nguyen *et al*., 2018; Nimrat *et al*., 2012; Toledo *et al*., 2019; Wang *et al*., 2008; Zokaeifar *et al*., 2012), but Arias-Moscoso *et al*. (2018) reported that the addition of probiotics did not improve the water quality nor productive response of *L. vannamei*. Meta-analysis showed that the probiotic effect on growth performance and shrimp survival depends on rearing conditions, method of administration, dosage, probiotic strain, shrimp species and production stage (Toledo *et al*., 2019). In this study, we demonstrated that *L. plantatum* T13, a beteriocinogenic bacterium isolated from traditional Vietnamese fermented cabbage (Nguyen *et al*., 2014b), could enhance shrimp growth. The crude bacteriocin extract from T13 is stable at 100-120°C and has strong and broad antimicrobial activity against food spoilage and animal pathogenic bacteria including diverse *Vibrio* species (Nguyen *et al*., 2014b). In addition, the combination of T13 with *Bacillus pumilus* B3.10.2B and *Bacillus cereus* D9 in probiotics formulation Biolobster 2 feed increased the growth rate and feed conversion ratio and resulted in increased survival after exposure to *V. owensii* as a pathogen in *P. ornatus* juveniles (Nguyen *et al*., 2014a). Nguyen *et al*. (2018) reported that T13 inhibited the growth of AHPND-causing *V. paraheamolytics* XN9, which was isolated from a AHPND outbreak in Ninh Thuan province, Vietnam in 2015. There was a report that the daily addition of probiotic *Bacillus fusiformis* could improve the survival and accelerate the metamorphosis of *P. monodon* and *L. vannamei* in larval shrimp rearing system with no water exchange (Guo *et al*., 2009).

Aquaculture has become one of major protein sources and requires high quality feed as well as some supplements to keep organisms healthy which favors growth (Farzanfar, 2006; Wang *et al*., 2008). Probiotics are a good candidate for facilitating feed utilization, digestion and growth of aquatic organisms (Farzanfar, 2006; Wang *et al*., 2008). Probiotics in the feed are commercially available and have been introduced to fish, shrimp and mollusks farming (Wang *et al*., 2008). *Lactobacillus* and *Bacillus* are potential probiotics applied in aquaculture (Farzanfar, 2006; Toledo *et al*., 2019). Dietary administration of T13 at concentrations of 10^9 CFU g^{-1} significantly improved the final weight and weight gain of shrimp, although FCR and SGR of T13 10^5 CFU g^{-1} were not statistically different from control (table 2). The effect of two probiotics *L. plantarum* T8 and T13 at the concentration of 10^8 CFU g^{-1} on body length and weight of *L. vannamei* studied by Nguyen *et al*. (2018) showed no significant differences (P>0.05) among treatments after 5 weeks. However, the shrimps from T8 treatment showed a significant difference (P<0.05) in body length and weight compared to the control after 12 weeks. The mixture of two probiotic strains, *B. subtilis* L10 and G1 at two different concentrations 10^5 and 10^8 CFU g^{-1} were previously performed on the growth

performance and digestive enzyme activity of juvenile white shrimp (Zokaeifar *et al*., 2012). The result showed that final weight, weight gain and digestive enzyme activity were significantly greater in shrimp fed with 10^5 and 10^8 CFU g^{-1} diets compared to the control group. The application of multiple-strain probiotics provided more effective in shrimp growth and overall health than single probiotic strains (Wang *et al*., 2019). The possible explanation for the improvement of shrimp growth could be related to the modulation of the intestinal microbiota of *L. vannamei* and the induction of digestive enzymes (Castex *et al*., 2008; Luis-Villasenor *et al*., 2013; Nimrat *et al*., 2012). Becerra-Dorame *et al.* (2012) evaluated the effect of autotrophic (AS) and heterotrophic (HS) microbial based systems on the digestive enzymatic activity of postlavae *L. vannamei*. According to their results, no significant differences were observed for trypsin, amylase and lipase activities between the AS and control group, whereas the shrimp from HS showed higher trypsin and amylase activities (Becerra-Dorame *et al*., 2012). Oujifard et al. 2012 conducted the experiments to evaluate the effects of different protein levels and sources of protein, growth performance and digestibility of Pacific white shrimp. The result revealed that weight gain increased when protein levels increased which was related to digestibility. The protein digestibility for a rice protein diet was distinctly lower than the fish meal diet , therefore, digestibility of protein was significantly affected by sources of protein (Oujifard *et al*., 2012). Highly significant differences (P<0.05) of total protein, protease and amylase activity were observed for *L. vanamei* fed diets supplemented with two probiotic *B. subtilis* strains for 8 weeks (Zokaeifar *et al*., 2012). In the present study, the slight increase of amylase activity (P>0.05) and significantly increase (P<0.05) of protease activity suggested that enhanced digestive activity was possibly caused by the presence of probiotic T13. It could be concluded that the increase of protease activity markedly contributed to weight gain and FCR (Table 2). Consequently, improvement in shrimp growth in treatment groups may be due to the concentrations of probiotics with their activity localized in the digestive systems of shrimp. Kewcharoen *et al.* (2019) demonstrated that high doses (10^9 CFU kg^{-1} diet) of probiotic *B. subtilis* AQAHBS001 provided positive effects on mucosal structure which plays important role in digestive activity. The increasing of height and width of the intestinal epithelial cell and villi help the digestion process by increasing absorption surface area (Kewcharoen *et al*., 2019). The results of electron microscopic revealed that the intestinal mucosa was tight and the epithelium cells showed an active secretory state in the probiotics group (Zuo *et al*., 2019). A diverse group of intestinal bacterial communities was observed in shrimp treated with and without probiotics and influenced the health of the host (Luis-Villasenor *et al*., 2013). The composition of the intestinal microbiota of shrimp treated with probiotics was distinctly different from control group by SSCP fingerprints (Luis-Villasenor *et al*., 2013). However, the effect of probiotics on intestinal microflora of *L. vannamei* was periodic, therefore, the microbial communities would tend to return to its original state (Zuo *et al*., 2019). The survival of probiotics strain in the shrimp digestive tract is an important feature. *B. subtilis* AQAHBS001 showed the ability to adhere, colonize and survive in outer surface areas, the surrounding water and the midgut of shrimp which was confirmed by qPCR (Kewcharoen *et al*., 2019). Probiotic *P. acidilactici* administered at a

concentration close to 10^7 CFU g^{-1} of feed was recovered around 10^4-10^5 CFU g^{-1} in shrimp gut after 2 h of feeding and decreased to 10^3 CFU g^{-1} after 6 h of feeding (Castex *et al*., 2008). This result suggested that the probiotic strain must be transient and may not adhere to the intestinal mucous of shrimp. However, de Vries *et al*. (2006) reported that *L. plantarum* has a proven ability to survive gastric transit and can colonize the intestinal tract of human and other mammals such as mice. Moreover, *L. plantarum* has a long history of natural occurrence and safe use in food products for human consumption such as plant, milk and meat products (de Vries *et al*., 2006). Several reports showed that different probiotic strains exhibit different results (Arias-Moscoso *et al*., 2018; Castex *et al*., 2008; Luis-Villasenor *et al*., 2013; Nimrat *et al*., 2012; Toledo *et al*., 2019; Zokaeifar *et al*., 2012). Therefore, screening and evaluation of potential probiotics are still challenging.

Conclusions

From our study, the probiotic T13 improved final weight, FCR and SGR of juvenile Pacific white shrimp but had no effect on survival rate. T13 could increase protease activity after feeding shrimp for 8 weeks and the appropriate concentration of probiotic T13 was 10^9 CFU g^{-1}.

Acknowledgements

We would like to thank Gong Kanchana 4, Sathing Phra, Songkhla province for the gift of shrimp. Special thank to Van Duy Nguyen for the gift of T13 strain. We also thank Rattana Satakij, Sukuman Sukumanant and Ismael Binsoh for technical assistance. This study was part of the Science classroom in University-affiliated school supported by Ministry of Science and Technology.

References

Arias-Moscoso, J. L., Espinoza-Barron, L. G., Miranda-Baeza, A., Rivas-Vega, M. E., & Nieves-Soto, M. (2018). Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zero water exchange. *Aquaculture Reports*, *11*, 47-52.

Becerra-Dorame, M. J., Martinez-Porchas, M., Martinez-Cordova, L. R., Rivas-Vega, M. E., Lopez-Elias, J. A., & Porchas-Cornejo, M. A. (2012). Production response and digestive enzymatic activity of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) intensively pregrown in microbial heterotrophic and autotrophic-based systems. *Scientific World Journal*, *2012*, 723654.

Buntin, N., Chanthachum, S., & Hongpattarakere, T. (2008). Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. *Songklanakarin Journal of Science and Technology*, *30*(Suppl.1), 141-148.

Castex, M., Chim, L., Pham, D., Lemaire, P., Wabete, N., Nicolas, J.-L., Schmidely, P., & Mariojouls, C. (2008). Probiotic *P. acidilactici* application in shrimp *Litopenaeus stylirostris* culture subject to vibriosis in New Caledonia. *Aquaculture*, *275*(1), 182-193.

de Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006). *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, *16*(9), 1018-1028.

Desriac, F., Defer, D., Bourgougnon, N., Brillet, B., Le Chevalier, P., & Fleury, Y. (2010). Bacteriocin as weapons in the marine aminal-associated bacteria warfare: inventory and potential applications as an aquaculture probiotic. *Marine drugs*, *8*, 1153-1177.

Dobson, A., Cotter, P. D., Ross, R. P., & Hill, C. (2012). Bacteriocin production: a probiotic trait? *Applied and Environmental Microbiology*, *78*(1), 1-6.

Dutta, T. K., Janan, M., Pahari, P. R., & Bhattacharya, T. (2006). The Effect of Temperature, pH, and Salt on Amylase in *Heliodiaptomus viduus* (Gurney) (Crustacea: Copepoda: Calanoida). *Turkish Journal of Zoology*, *30*(2), 187-195.

Farzanfar, A. (2006). The use of probiotics in shrimp aquaculture. *FEMS Immunol Med Microbiol*, *48*(2), 149-158.

Flegel, T. W. (2006). Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture*, *258*(1-4), 1-33.

Gillor, O., Etzion, A., & Riley, M. A. (2008). The dual role of bacteriocins as anti- and probiotics. *Applied Microbiology and Biotechnology*, *81*(4), 591-606.

Guo, J.-J., Liu, K.-F., Cheng, S.-H., Chang, C.-I., Lay, J.-J., Hsu, Y.-O., Yang, J.-Y., & Chen, T.-I. (2009). Selection of probiotic bacteria for use in shrimp larviculture. *Aquaculture Research*, *40*, 609-618.

Kewcharoen, W., & Srisapoome, P. (2019). Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). *Fish & Shellfish Immunology*, *94*, 175-189.

Lebel, L., Mungkung, R., Gheewala, S. H., & Lebel, P. (2010). Innovation cycles, niches and sustainability in the shrimp aquaculture industry in Thailand. *Environmental Science & Policy*, *13*(4), 291-302.

Luangthuvapranit, C., & Srichai, N. (2007). Evaluation of self-prepared test kit for outdoor dissolved oxygen determination. *Songklanakarin Journal of Science and Technology*, *29*(1), 100-108.

Luis-Villasenor, I. E., Castellanos-Cervantes, T., Gomez-Gil, B., Carrillo-Garcia, A. E., Campa-Cordova, A. I., & Ascencio, F. (2013). Probiotics in the intestinal tract of juvenile whiteleg shrimp *Litopenaeus vannamei*: modulation of the bacterial community. *World Journal of Microbiology and Biotechnology*, *29*(2), 257-265.

Luis-Villasenor, I. E., Macis-Rodriguez, M. E., Gomez-Gil, B., Ascencio-Valle, F., & Campa-Cordova, A. I. (2011). Beneficial effects of four *Bacillus* strains on the larval cultivation of *Litopenaeus vannamei*. *Aquaculture*, *321*(1-2), 136-144.

Martinez Cruz, P., Ibanez, A. L., Monroy Hermosillo, O. A., & Ramirez Saad, H. C. (2012). Use of probiotics in aquaculture. *ISRN microbiology*, *2012*, 916845.

Nguyen, T. T. G., Nguyen, T. C., Leelakriangsak, M., Pham, T. T., Pham, Q. H., Lueangthuwapranit, C., & Nguyen, V. D. (2018). Promotion of *Lactobacillus plantarum* on growth and resistance against acute hepatopancreatic necrosis disease pathogens in white-leg shrimp (*Litopenaeus vannamei*). *The Thai Journal of Veterinary Medicine*, *48*(1), 19-28.

Nguyen, V. D., Pham, T. T., Nguyen, T. H., Nguyen, T. T., & Hoj, L. (2014a). Screening of marine bacteria with bacteriocin-like activities and probiotic potential for ornate spiny lobster (*Panulirus ornatus*) juveniles. *Fish Shellfish Immunology*, *40*(1), 49-60.

Nguyen, V. D., Pham, T. T., & Pham, N. M. Q. (2014b). Two novel strains of bacteriocin-producing *Lactobacillus plantarum* and their application as biopreservative in chill-stored fresh cobia meat. *Journal of Pure and Applied Microbiology*, *8*(2), 1547-1557.

Nimrat, S., Suksawat, S., Boonthai, T., & Vuthiphandchai, V. (2012). Potential *Bacillus* probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*). *Veterinary Microbiology*, *159*(3-4), 443-450.

Oujifard, A., Seyfabadi, J., Abedian Kenari, A., & Rezaei, M. (2012). Growth and apparent digestibility of nutrients, fatty acids and amino acids in Pacific white shrimp, *Litopenaeus vannamei*, fed diets with rice protein concentrate as total and partial replacement of fish meal. *Aquaculture*, *342-343*, 56-61.

Pineiro, M., & Stanton, C. (2007). Probiotic Bacteria: Legislative Framework Requirements to Evidence Basis. *The Journal of Nutrition*, *137*(3), 850S-853S.

Shimogaki, H., Takeuchi, K., Nishino, T., Ohdera, M., Kudo, T., Ohba, K., Iwama, M., & Trie, M. (1991). Purification and Properties of a Novel Surface-active Agent- and Alkaline-resistant Protease from *Bacillus* sp. Y. *Agricultural and Biological Chemistry*, *55*(9), 2251-2258.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P. V., Sritunyalucksana, K., Flegel, T. W., & Itsathitphaisarn, O. (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, *452*, 69-87.

Toledo, A., Frizzo, L., Signorini, M., Bossier, P., & Arenal, A. (2019). Impact of probiotics on growth performance and shrimp survival: A meta-analysis. *Aquaculture*, *500*, 196-205.

Vieira, F. d. N., Jatobá, A., Mouriño, J. L. P., Vieira, E. A., Soares, M., da Silva, B. C., Seiffert, W. Q., Martins, M. L., & Vinatea, L. A. (2013). In vitro selection of bacteria with potential for use as probiotics in marine shrimp culture. *Pesquisa Agropecuaria Brasileira*, *48*(8), 998-1004.

Wang, Y.-C., Hu, S.-Y., Chiu, C.-S., & Liu, C.-H. (2019). Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. *Fish & Shellfish Immunology*, *84*, 1050-1058.

Wang, Y.-B., Li, J.-R., & Lin, J. (2008). Probiotics in aquaculture Challenges and outlook. *Aquaculture*, *281*, 1-4.

Zokaeifar, H., Balcazar, J. L., Saad, C. R., Kamarudin, M. S., Sijam, K., Arshad, A., & Nejat, N. (2012). Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *33*(4), 683-689.

Zuo, Z.-H., Shang, B.-J., Shao, Y.-C., Li, W.-Y., & Sun, J.-S. (2019). Screening of intestinal probiotics and the effects of feeding probiotics on the growth, immune, digestive enzyme activity and intestinal flora of *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *86*, 160-168.