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Research Article

Bacterial Population, Activity of Enzymes and Growth Rate of Pacific White Shrimp Larvae Administered *Pseudoalteromonas piscicida* and Mannan-oligosaccharides through Bio-encapsulation of *Artemia* sp.

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Abstract

Background and Objective: The application of probiotics, prebiotics and synbiotics is commonly applied in juvenile and adult shrimp administered through artificial feed, while in larvae, it has still been limited. This study aimed to evaluate growth performance, the total intestinal bacteria, the activity of enzymes and the survival rate of Pacific white shrimp (*Litopenaeus vannamei*) administered *Pseudoalteromonas piscicida* 1Ub, mannan-oligosaccharides and synbiotic (the combination of *P. piscicida* 1Ub and mannan-oligosaccharides) through bio-encapsulation of *Artemia* sp. **Materials and Methods:** Bio-encapsulation of *Artemia* sp. was done by adding *P. piscicida* 1Ub 10^6 CFU mL^{-1}, mannan-oligosaccharides 12 mg L^{-1} and synbiotic (*P. piscicida* 1Ub 10^6 CFU mL^{-1} and mannan-oligosaccharides 12 mg L^{-1}) to the rearing medium of *Artemia* sp., for 4 h. The administration of the enriched *Artemia* sp. to the shrimp larvae was done from mysis 3 to Post Larvae (PL) 12. The body length and the body weight of Pacific white shrimp larvae were observed at the beginning and the end of the study, while RNA/DNA ratio, the activity of enzymes, survival rate and total bacteria of shrimp larvae were analyzed at the end of the study. **Results:** The results showed that daily growth rate, absolute length, RNA/DNA ratio, the activity of enzymes, survival rate and total bacteria of shrimp larvae administered probiotic, prebiotic and synbiotic were higher ($p<0.05$) than the control. The synbiotic treatment gave the best results in daily growth rate (24.39±0.31%), absolute length (13.00±0.50 mm), RNA/DNA ratio (0.6369±0.0094 μg mL^{-1}), the activity of enzymes (protease 0.033±0.0007, lipase 0.047±0.0010, amylase 0.853±0.008, mannanase 0.148±0.004 U mL^{-1} min^{-1}), survival rate (92.67±1.26%) and total bacteria (6.7×10^7 CFU $larvae^{-1}$). **Conclusion:** The administration of *P. piscicida* 1Ub, mannan-oligosaccharides and synbiotic through bio-encapsulation of *Artemia* sp., effectively improved the growth performance of Pacific white shrimp larvae with the best results demonstrated by the synbiotic treatment.

Key words: Probiotic, prebiotic, synbiotic, *Artemia* sp., Pacific white shrimp

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

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei*) is one of major export commodities from Indonesia from the fishery sector. Indonesia is the 3rd largest shrimp exporter in the world after India and Equador, with an export volume of 181,351 t[1]. The development of Pacific white shrimp production must be supported by the sustainable supply of a high quality shrimp larvae. The high quality larvae would result in a good growth and a high survival rate. The application of probiotics, prebiotics and synbiotics is an alternative that could be used to increase the growth and the survival rate of the shrimp.

A probiotic is a beneficial microbe for the cultivated organism, because it could modify the microbial community, improve the nutritional value, improve the host's response to disease, improve the environmental quality[2] and could improve the immune response[3]. The results of previous studies have proven the probiotic's success in improving the shrimp's growth, survival rate, immune response and resistance[4-7]. In this study, the probiotic *Pseudoalteromonas piscicida* 1Ub which was proven to be able to inhibit the growth of the pathogenic bacteria *Vibrio harveyi* and increase the giant tiger prawn larvae's survival rate[8].

The role of probiotic bacteria could be improved through the application of a prebiotic, a feed material that cannot be digested and is beneficial for the host because it stimulates the growth and the activity of certain bacteria in the intestines and thus improving the host's health[9]. A number of studies have demonstrated that prebiotics can improve growth, survival rate, feed digestibility, feed efficiency, the composition of microflora in the intestines, inhibit the growth of pathogens and improve the shrimp's immune system[10-12]. The prebiotic used in this study was mannan-oligosaccharides (MOS) which has been demonstrated to improve the growth and the survival rate of Pacific white shrimp juvenile.

If a probiotic and a prebiotic are combined in a single product (synbiotic), the benefits will increase[13]. The use of synbiotics have been demonstrated to improve growth, survival rate, immune response and resistance in aquatic organisms, including in the European lobster larvae, *Homarus gammarus* L.[14,15] and Pacific white shrimp, *L. vannamei*[16-18].

In addition, the application of probiotics, prebiotics and synbiotics is commonly applied in the juvenile and the adult shrimp administered through artificial feed[10,16-18], while in larvae, it has been limited to the larvae of *H. gammarus* L.[14,15] and *L. vannamei* larvae with the probiotic *Vibrio alginolyticus* SKT-b Rf^R and the prebiotic oligosaccharide derived from sweet potato var., *sukuh* extract[19]. The application of probiotics, prebiotics and synbiotics since the larval stadia is important to produce the high quality shrimp larvae with the high growth rate and resistance against certain diseases (specific pathogen resistance), so that when the shrimp larvae are stocked in grow out ponds, they already have the better growth performance and immune response to face attacks from the various pathogens found in the pond. In this study, the application of probiotic, prebiotic and synbiotic in Pacific white shrimp larvae was conducted through the enrichment of *Artemia*, the main natural feed for shrimp larvae, because it has the appropriate size for larvae, has a high nutritional value and is easy to be digested. This study aimed to evaluate total bacteria, the activity of enzymes and growth performance of Pacific white shrimp larvae administered probiotic *P. piscicida* 1Ub, prebiotic MOS and synbiotic (a combination between probiotic *P. piscicida* 1Ub and prebiotic MOS) through bio-encapsulation of *Artemia* sp.

MATERIALS AND METHODS

Probiotic and prebiotic preparation: The probiotic used was *P. piscicida* 1Ub, isolated from the nauplii of Pacific white shrimp[8]. The *P. piscicida* 1Ub bacterial isolate was marked with the antibiotic rifampicin (1Ub Rf^R) as a molecular marker. The *P. piscicida* 1Ub Rf^R cells were cultured in seawater complete (SWC) slant agar medium (0.5 g bacto peptone, 0.1 g yeast extract, 0.3 mL glycerol, 1.5 g bacto agar, 75 mL seawater and 25 mL distilled water) and were incubated at 29°C for 24 h. Then the bacterial cells were inoculated into SWC broth medium and were incubated in a water bath shaker (29°C, 140 rpm) for 18 h.

The prebiotic used was Bio-MOS (Alltech Inc., KY USA) which contained mannan-oligosaccharides (MOS) derived from the cell walls of *Saccharomyces cerevisiae* with a composition of 30% crude protein, 1.4% crude fat and 13% crude fiber.

Rearing medium and experimental animal preparation: The rearing medium for the Pacific white shrimp larvae were 12 aquariums ($60 \times 30 \times 35$ cm^3), which were equipped with aeration equipment and heater. The aquariums were filled with 10 L disinfected seawater with a salinity of 30 ppt. The experimental animals were Pacific white shrimp larvae (mysis 3) and were stocked at a density of 200 individuals per aquarium[4].

Enrichment of *Artemia* sp.: *Artemia* cysts (2 g L^{-1} seawater) were hatched in strongly aerated seawater (30 ppt), then it were harvested after 24 h. The enrichment of *Artemia* sp. was

conducted on instar 2 stadia of *Artemia* sp. (approximately 4 h after being harvested) using a plastic container filled with 1 L seawater (30 ppt). The density of *Artemia* sp. in each container was 100 individuals mL^{-1} [7]. The enrichment was conducted by adding the probiotic *P. piscicida* 1Ub Rf^R at a concentration of 10^6 CFU mL^{-1}, 12 mg L^{-1} prebiotic MOS and a combination between 10^6 CFU mL^{-1} probiotic *P. piscicida* 1Ub Rf^R and 12 mg L^{-1} MOS (synbiotic) into each enrichment container of *Artemia* sp. The enrichment was conducted for 4 h[20]. And then the *Artemia* were harvested using a plankton net and were washed with the disinfected seawater, then those were fed to the Pacific white shrimp larvae at a dose of 8-10 individuals per larvae[21] and the rest were stored in a refrigerator at 4°C for further use on the same day. During the rearing period, 5-10% of the rearing medium water was siphoned and was replaced every 3 days.

Experimental design: *Artemia* were fed to the larvae 5 times a day at 06.00 am, 10.00 am, 02.00 pm, 06.00 pm and 10.00 pm. This study was conducted through the completely randomized design with four treatments and three repeats. The treatments were (1) Pacific white shrimp larvae fed *Artemia* without any probiotic, prebiotic and synbiotic enrichment (control), (2) Pacific white shrimp larvae fed *Artemia* enriched with probiotic *P. piscicida* 1Ub Rf^R at a concentration of 10^6 CFU mL^{-1} (probiotic), (3) Pacific white shrimp larvae fed *Artemia* enriched with prebiotic MOS 12 mg L^{-1} (prebiotic) and (4) Pacific white shrimp larvae fed *Artemia* enriched with the combination between the probiotic *P. piscicida* 1Ub Rf^R at a concentration of 10^6 CFU mL^{-1} and 12 mg L^{-1} the prebiotic MOS (synbiotic).

Survival rate and growth of Pacific white shrimp larvae: The Pacific white shrimp larvae's survival rate was calculated at the end of the study using the formula according to Dehaghani *et al.*[22]. The growth parameters observed were Daily Growth Rate (DGR) and absolute length (L). The DGR was calculated using the formula according to Nurhayati *et al.*[18] and the absolute length growth (L) was calculated using the formula according to Dehaghani *et al.*[22].

To support the growth parameter data, the calculation of RNA/DNA ratio of the shrimp larvae was done using the gene quant calculator. The extraction of the shrimp larvae RNA and DNA was conducted by placing larvae (n = 3 individuals) from each treatment into 1.5 mL tubes which had been filled with 200 µL isogen on ice, then the sample was ground until it was completely macerated. After all tissues had been macerated, 400 µL isogen was added, then the mixture was stored at a room temperature for 5 min for lysis, then 200 µL chloroform ($CHCl_3$) was added, the sample was homogenized using a vortex for 15 sec and was stored at a room temperature for 2-3 min. The lysis product was centrifuged at 10,000 rpm for 15 min until three layers are formed: The supernatant (clear) on the top layer was chloroform+RNA, the second layer was protein and the pellet was phenol+DNA (blue). The supernatant (chloroform+RNA) and the pellet (phenol+DNA) were collected, then each layer was moved to a new tube which had been filled with 400 µL isopropanol, it was homogenized and stored at a room temperature for 5-10 min, then each layer was centrifuged at 4°C and 10,000 rpm for 15 min. The supernatant was discarded and the pellet remaining at the bottom of the tube was added with 1 mL cold ethanol 70%. Then this was centrifuged again at 4°C and 10,000 rpm for 15 min, the supernatant was discarded and the pellet was air dried. After dried, the pellet was added with 50 µL diethylpyrocarbonate (DEPC), then it was homogenized with a vortex and was stored on ice. The concentrations of the genome's RNA and DNA were then measured using the gene quant. The concentration of RNA and DNA was the result of the gene quant reading multiplied with dilution factor, while RNA/DNA ratio (µg mL^{-1}) was calculated by dividing the RNA concentration by the genome's DNA concentration[23].

Bacterial population: Total bacterial count and total probiotic *P. piscicida* 1Ub Rf^R in the shrimp larvae were enumerated through the spread plate method[24]. The shrimp larvae from each treatment at a weight of 0.1 g (5-6 shrimps) were macerated and were homogenized in 0.9 mL phosphate buffer saline (0.8% NaCl, 0.15% K_2HPO_4, 0.02% Na_2HPO_4 and 0.02% KCl). The serial dilution was performed 10 times, the bacterial suspension from the serial dilution was then spread onto the SWC plate agar medium for the enumeration of total bacteria and onto SWC plate agar+rifampicin (50 µg mL^{-1}) medium for the enumeration of total probiotic *P. piscicida* 1Ub Rf^R.

Activity of enzymes: The activity of Pacific white shrimp larvae's enzymes analyzed, included the activity of protease, lipase, amylase and mannanase. The shrimps used for the analysis were 25-30 shrimps sample (0.5 g $sample^{-1}$). The procedure for analyzing the activity of protease and amylase followed the method constructed by Bergmeyer *et al.*[25], the procedure for analyzing the activity of lipase followed the method constructed by Borlongan[26], while the procedure for analyzing the activity of mannanase followed the method constructed by Hossain *et al.*[27].

Statistical analysis: The data of the daily growth rate, the absolute length, the RNA/DNA ratio, the bacterial population,

the activity of enzymes and the survival rate of Pacific white shrimp were analyzed through ANOVA. Differences among treatments were analyzed through the Duncan's test at a confidence interval 95%. Those statistical analysis were operated by SPSS 16 program.

RESULTS

Survival rate and growth performance: The application of *Artemia* sp., enriched with probiotic, prebiotic and synbiotic on Pacific white shrimp larvae (mysis 3 to PL 12) had a significant effect ($p<0.05$) on the larvae's survival rate (Fig. 1). The highest survival rate ($p<0.05$) was found in the synbiotic treatment (92.67±1.26%), followed by the probiotic treatment (88.67±1.76%), the prebiotic treatment (87.83±1.76%) and the lowest was found in the control treatment (84.17±1.04%).

The application of probiotic, prebiotic and synbiotic through *Artemia* sp. in Pacific white shrimp larvae had a significant effect ($p<0.05$) on the DGR and absolute length, but only the application of probiotic and synbiotic had a significant effect ($p<0.05$) on the RNA/DNA ratio (Table 1). The highest DGR value ($p<0.05$) was found in the synbiotic treatment (24.39±0.31%), followed by the probiotic treatment (21.96±0.24%), prebiotic treatment (21.30±0.39%) and the lowest was in the control treatment (18.54±0.29%). Similar results were found in the absolute length values, the highest value ($p<0.05$) was found in the synbiotic treatment (13.00±0.50 mm), followed by the probiotic treatment (11.33±0.29 mm) and the prebiotic treatment (10.83±0.58 mm), while the lowest was in the control treatment (8.83±0.76 mm). The RNA/DNA ratio for the Pacific white shrimp larvae during the rearing period ranged between 0.2834-0.6369 μg mL^{-1}. The highest RNA/DNA ratio ($p<0.05$) was demonstrated by the synbiotic treatment (0.6369±0.0094 μg mL^{-1}), followed by the probiotic treatment (0.4207±0.0459 μg mL^{-1}), while that in the prebiotic treatment (0.3201±0.0349 μg mL^{-1}) was not significantly different ($p<0.05$) from the control treatment (0.2834±0.0269 μg mL^{-1}).

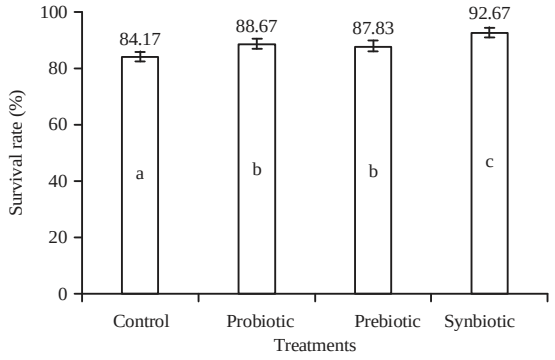

Fig. 1: Survival rate of Pacific white shrimp larvae given the probiotic *Pseudoalteromonas piscicida* 1Ub Rf^R, prebiotic MOS and synbiotic (combination between *Pseudoalteromonas piscicida* 1Ub Rf^R and MOS) through the enrichment of *Artemia* sp., from mysis 3 to PL 12. Different letters within the bars indicate significant different results at $p<0.05$

Table 1: Growth performance of Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through the enrichment of *Artemia* sp.

Treatments	DGR (%)	Absolute length (mm)	RNA/DNA ratio (μg mL^{-1})
Control	18.54±0.29^a	8.83±0.76^a	0.2834±0.0269^a
Probiotic	21.96±0.24^c	11.33±0.29^b	0.4207±0.0459^b
Prebiotic	21.30±0.39^b	10.83±0.58^b	0.3201±0.0349^a
Synbiotic	24.39±0.31^d	13.00±0.50^c	0.6369±0.0094^c

DGR: Daily growth rate. Different superscript letters in the same column indicate significant different results at $p<0.05$

Bacterial population: The bacterial population values inside the Pacific white shrimp larvae's bodies in the treatment with the application of the probiotic and synbiotic were higher ($p<0.05$) than in those treated with the prebiotic and the control. The total bacterial count in the synbiotic treatment was 6.7×10^7 CFU $larvae^{-1}$, followed by the probiotic treatment (3.87×10^7 CFU $larvae^{-1}$), the prebiotic treatment (3.74×10^5 CFU $larvae^{-1}$) and the control treatment (2.42×10^5 CFU $larvae^{-1}$). In addition, the probiotic *P. piscicida* 1Ub Rf^R was able to survive and colonize in the Pacific white shrimp larvae. The population of the probiotic *P. piscicida* 1Ub Rf^R in the bodies of the Pacific white shrimp larvae fed the probiotic treatment was 5.84×10^5 CFU $larvae^{-1}$ and that given the synbiotic treatment was 4.75×10^6 CFU $larvae^{-1}$ (Table 2).

The results of the sequencing of the dominant bacteria in the bodies of the Pacific white shrimp larvae using the primer 16S rRNA revealed five species of bacteria: *Staphylococcus pasteuri*, *Tenacibaculum mesophilum*, *P. piscicida*, *V. alginolyticus* and *Staphylococcus warneri*. The *S. pasteuri* was predominantly found in the bodies of the shrimp larvae given the probiotic *P. piscicida* 1Ub Rf^R and the

Table 2: Total bacteria and total *Pseudoalteromonas piscicida* 1Ub Rf^R in the bodies of Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through the enrichment of *Artemia* sp.

Treatments	Total bacteria (CFU $larvae^{-1}$)	Total *Pseudoalteromonas piscicida* 1Ub Rf^R (CFU $larvae^{-1}$)
Control	2.42×10^{5a}	-^{a}
Probiotic	3.87×10^{7b}	5.84×10^{5b}
Prebiotic	3.74×10^{5a}	-^{a}
Synbiotic	6.70×10^{7c}	4.75×10^{6c}

Different superscript letters in the same column indicate significant different results at $p<0.05$

Table 3: Activity of enzymes in Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through the enrichment of *Artemia* sp.

	Activity of enzymes (U mL^{-1} min^{-1})			
Treatments	Protease	Lipase	Amylase	Mannanase
Control	0.011 ± 0.0004^{a}	0.037 ± 0.0015^{a}	0.576 ± 0.004^{a}	0.070 ± 0.001^{a}
Probiotic	0.022 ± 0.0044^{b}	0.041 ± 0.0005^{c}	0.788 ± 0.006^{c}	0.119 ± 0.052^{ab}
Prebiotic	0.013 ± 0.0016^{a}	0.044 ± 0.0005^{b}	0.613 ± 0.008^{b}	0.109 ± 0.004^{ab}
Synbiotic	0.033 ± 0.0007^{c}	0.047 ± 0.0010^{d}	0.853 ± 0.008^{d}	0.148 ± 0.004^{b}

Different superscript letters in the same column indicate significant different results at $p<0.05$

synbiotic. The *T. mesophilum* was predominantly found in the bodies of the shrimp larvae given the prebiotic MOS, the probiotic *P. piscicida* 1Ub Rf^R and the control. The *P. piscicida* was predominantly found in the shrimp larvae given the probiotic *P. piscicida* 1Ub Rf^R and the synbiotic. The *V. alginolyticus* and *S. warneri* were predominantly found in the shrimp larvae given the probiotic *P. piscicida* 1Ub Rf^R.

Activity of enzymes: The activity of enzymes in Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through bio-encapsulation of *Artemia* sp., demonstrated varied values among treatments (Table 3). The protease activities in the synbiotic and probiotic treatment were higher ($p<0.05$) than those in the prebiotic treatment and the control. The lipase and amylase activities in the synbiotic treatment were higher ($p<0.05$) than the other treatments and the control. Similar results were found in the values of mannanase activity; the synbiotic treatment had a higher value ($p<0.05$) than the control, but the mannanase activity in the synbiotic treatment was not significantly different ($p>0.05$) from the probiotic and prebiotic treatment.

DISCUSSION

The growth rate and the survival rate of Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through bio-encapsulation of *Artemia* sp. were higher than the control. This is estimated to be related to the ability of probiotic *P. piscicida* 1Ub Rf^R and prebiotic MOS in modulating the growth and the activity of beneficial microflora in the digestive tract of Pacific white shrimp larvae. They could help increase the feed digestibility, affecting the larval growth and the survival rate. This was demonstrated by the high total bacteria and total *P. piscicida* 1Ub Rf^R in the bodies of the Pacific white shrimp larvae given the probiotic, prebiotic and synbiotic through bio-encapsulation of *Artemia* sp. Endogenous bacteria and the probiotic bacteria *P. piscicida* 1Ub Rf^R in the bodies of the Pacific white shrimp larvae contributed in producing the enzymes needed for the digestion, so the feed digestibility, growth performance and survival rate of Pacific white shrimp larvae given the treatments were better than those not given the treatment (control).

Probiotics are live microbial agents which can influence the survival rate, growth and health status of aquatic animals. De Preter *et al.*[28] explained that the survival rate, colonization and beneficial effects of exogenous probiotics can be increased and improved by the simultaneous addition of prebiotics known as synbiotics.

The application of the synbiotic (the combination between *P. piscicida* 1Ub Rf^R and MOS) through the bio-encapsulation of *Artemia* sp. in Pacific white shrimp larvae (mysis 3 to PL 12) resulted in the best DGR, absolute length and RNA/DNA ratio among other treatments and the control. These results were in line with the results shown by the micro-encapsulated synbiotic, which was the combination between *Bacillus* NP5 Rf^R and mannan-oligosaccharides in *L. vannamei*[29], the combination between the probiotic *V. alginolyticus* SKT-b Rf^R and the oligosaccharide extracted from sweet potato var., *sukuh* in *L. vannamei* larvae[19], the combination between the probiotic *V. alginolyticus* SKT-b Rf^R and the oligosaccharide extracted from sweet potato var., *sukuh* in *L. vannamei*[18] and the combination between *Bacillus* spp. and mannan-oligosaccharides in the European lobster larvae[15].

The application of the synbiotic (the combination between *P. piscicida* 1Ub Rf^R and MOS) through the bio-encapsulation of *Artemia* sp. in Pacific white shrimp larvae also resulted in the best Survival Rate (SR) among treatments and the control. Some studies have demonstrated that synbiotics could improve the survival rate of aquatic organisms, e.g., the application of the synbiotic (the combination between the probiotic *V. alginolyticus* SKT-b and oligosaccharide extracted from sweet potato var., *sukuh*) through the enrichment of *Artemia* sp., could improve the survival rate of Pacific white shrimp larvae[19] and the addition of the synbiotic (the combination between the probiotic *Enterococcus faecium* and the prebiotic fructooligosaccharide) in feed could improve the survival rate of *Carassius auratus* gibelio juvenile[30]. It was caused by an improvement on immunity level of Pacific white shrimp larvae induced by the application of synbiotic. An increase in immunity level will lead to a better protection of the host against a pathogenic infection and environmental stress. Talas and Gulhan[31] stated that an increase in immunity level could protect shrimp against the pathogenic infection (white spot disease).

Based on the data of total bacteria and total probiotic *P. piscicida* 1Ub Rf^R count in the bodies of the Pacific white shrimp larvae, it could be seen that the application of the synbiotic resulted in a higher total bacterial count and total probiotic *P. piscicida* 1Ub Rf^R count than the other treatments and the control. The high total bacterial count and total probiotic *P. piscicida* 1Ub Rf^R count in the bodies of the Pacific white shrimp larvae given the synbiotic treatment was possible due to the probiotic *P. piscicida* 1Ub Rf^R ability to survive and colonize in the intestinal tract of Pacific white shrimp larvae, because the probiotic *P. piscicida* 1Ub Rf^R which was used in this study was bacteria isolated from the nauplii of Pacific white shrimp[8] and the ability to utilize mannan-oligosaccharides was caused by the mannanase enzyme produced by the probiotic *P. piscicida* 1Ub Rf^R bacteria. Similar results were reported by Widanarni *et al.*[7] who stated that the administration of various doses of the probiotic *V. alginolyticus* SKT-b through *Artemia* could increase the total *Vibrio* count in the bodies of the giant tiger prawn post-larvae and the application of the micro-encapsulated synbiotic (*Bacillus* NP5 Rf^R and oligosaccharide) could increase the bacterial population in the intestines of Pacific white shrimp up to log 9 CFU g^{-1} [32].

The results of the sequencing of the dominant bacteria in the bodies of the Pacific white shrimp larvae using the primer 16S rRNA[33] revealed that there were five bacterial species: *S. pasteuri*, *T. mesophilum*, *P. piscicida*, *V. alginolyticus* and *S. warneri*. These five species of bacteria are commonly found in organisms that live in seawater. The *T. mesophilum* was isolated from sea sponges and green algae[34]. The *S. pasteuri* and *S. warneri* have potential as probiotics that could influence growth and survival rate in *L. vannamei*[35], while *P. piscicida* and *V. alginolyticus* have been known and applied as probiotics to improve the growth and survival rate of giant tiger prawn and Pacific white shrimp[8,18].

The presence of the probiotic bacteria accumulated in *Artemia* sp., a natural feed could increase the exogenous enzyme production in the bodies of Pacific white shrimp larvae. The probiotic *P. piscicida* 1Ub Rf^R can produce a number of exogenous enzymes, including protease, lipase, amylase and mannanase. The probiotic *P. piscicida* 1Ub Rf^R ability to produce a number of exogenous enzymes allows the activity of enzymes in Pacific white shrimp larvae that had been given the treatments to be higher than the control. According to Wang *et al.*[36], probiotics are able to produce a number of exogenous enzymes for the digestion, including amylase, protease, lipase and cellulase. Tzuc *et al.*[37] reported that *Pseudoalteromonas* sp., isolated from the stomach, intestines and hepatopancreas of Pacific white shrimp could produce amylase, lipase and chitinase. Protease is able to hydrolyze protein into peptides and bacteria produce peptidase that breaks down peptides into amino acids that are needed for the metabolism. Amylase can hydrolyze amylum and help the digestion in organisms. Lipase is an enzyme that basically plays a role in the hydrolysis of fats, monoglycerides, diglycerides and triglycerides to produce free fatty acids and glycerol[38]. The main function of lipase is to digest fats, maintain the function of the gall bladder, maintain the balance of electrolytes in the body, maintain an optimum cell permeability, thus allowing nutrients that are needed to enter the cell to facilitate the metabolism.

The digestion in the stomach and intestines are effective because of the high activity of enzymes. The *P. piscicida* 1Ub Rf^R supplemented to *Artemia* sp. has a function as a supplier of exogenous enzymes and helps the process of the feed breaking down into micro molecules that can be easily absorbed, so that the digestive system of Pacific white shrimp larvae becomes more effective in energy expenditure for the digestive process. The energy that would have been expended is reduced and the energy could be used for the growth instead. The feed materials that have been broken down into simple molecules are then absorbed by the intestines, enter the blood flow are distributed to tissues throughout the body and enter cells[39]. Inside the cells, glucose will be oxidized to produce energy, while protein and fat are retained inside the body tissues, then those are used for improving the growth performance. Liu *et al.*[40] stated that the increase in the growth of aquatic animals given probiotic could be linked to the increased digestive activities by enzymatic activity and vitamin synthesis which could improve digestibility and weight gain.

Supplementation of the prebiotic mannan-oligosaccharides in *Artemia* sp., indirectly adds the amount of exogenous enzymes in the bodies of Pacific white shrimp larvae, provides additional energy and nutrition for the natural bacteria in the bodies of the larvae to survive and produce more exogenous enzymes, making the digestion in Pacific white shrimp larvae to be more effective. This can be seen from the high total bacterial count and the activity of enzymes in Pacific white shrimp larvae given the synbiotic treatment. The prebiotic mannan-oligosaccharides used in this study was extracted from the cell walls of the yeast *S. cerevisiae* with a composition of 30% crude protein, 1.4% crude fat and 13% crude fiber.

Application of the synbiotic (the combination between *P. piscicida* 1Ub Rf^R and mannan-oligosaccharides) through the enrichment of *Artemia* sp., would create a synergy inside the Pacific white shrimp larvae's body. This can be seen from the total bacterial count and the activity of enzymes (protease,

lipase and amylase) of Pacific white shrimp larvae fed the synbiotic which were higher than those given the probiotic or prebiotic in single administration. The prebiotic mannan-oligosaccharides administered could be directly utilized by the probiotic *P. piscicida* 1Ub Rf^R or could be utilized by the natural bacteria found in the bodies of Pacific white shrimp larvae. The probiotic *P. piscicida* 1Ub Rf^R ability in utilizing mannan-oligosaccharides is closely related to the probiotic *P. piscicida* 1Ub Rf^R ability to produce mannanase. This allows the administration of the synbiotic through bio-encapsulation of *Artemia* sp., resulting in the maximum activity of enzymes and the growth performance in Pacific white shrimp larvae in this study. Similar results were also reported by Zokaeifar *et al*.[6], the administration of the probiotic *Bacillus subtilis* could increase digestive enzymes in Pacific white shrimp and the administration of the synbiotic (the combination between the probiotic *Enterococcus faecium* and the prebiotic fructooligosaccharide) could improve the activity of digestive enzymes in the carp juvenile[22].

CONCLUSION

The application of the probiotic *P. piscicida* 1Ub Rf^R, the prebiotic mannan-oligosaccharides and the combination between the probiotic *P. piscicida* 1Ub Rf^R and the prebiotic mannan-oligosaccharides (synbiotic) through bio-encapsulation of *Artemia* sp., could improve the growth performance, total bacteria, the activity of enzymes and survival rate of Pacific white shrimp larvae with the best results demonstrated by the synbiotic treatment.

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