



The Effect of Encapsulated Probiotic to Performance of Vannamei Shrimp (*Litopenaeus vannamei* Boone, 1931)

Yuli Andriani ^{a*}, Rusky I. Pratama ^a and Ratu Safitri ^b

^a *Department of Fisheries, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, West Java, Indonesia.*

^b *Department of Microbiology, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, West Java, Indonesia.*

Authors' contributions

This work was carried out in collaboration among all authors. Author YA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RIP managed the literature searches and writing, and author RS managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Feed is a very important factor in vannamei shrimp culture. The initial stage of stocking is the cultivation stage requiring a higher percentage of feed. The application of probiotics in intensive shrimp culture is known to have a significant effect on the survival of vannamei shrimp. Encapsulation can increase the viability of probiotic bacteria compared to free cells without encapsulation. Research has been carried out on the provision of encapsulated probiotic consortia in vannamei shrimp culture. The purpose of this study was to observe the effect of the selected probiotic consortium on the performance of vannamei shrimp. The research method used is the experimental method and the data obtained were analyzed descriptively. The observed parameters in this study were specific growth rate, survival rate and feed conversion ratio. The results showed that the fifth consortium of Bacillus and Lactobacillus bacteria could increase the growth rate of weight and length of vannamei shrimp (0.62% and 9.81%), survival rate (85.42%) and feed conversion ratio (1.08).

*Corresponding author: Email: yuliyusep@yahoo.com, yuli.andriani@unpad.ac.id;

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1. INTRODUCTION

Shrimp has a great prospect as a source of nutrient for humans, since shrimp is considered as one of the best sources for high-quality protein. In Indonesia, shrimp is the mainstay of non-oil export commodities. The production of aquaculture from crustaceans in 2010 consisted of 29.4% in fresh waters and 70.6% from marine waters which was dominated by the production of white shrimp (*Litopenaeus vannamei*) [1],2]. Intensive farming systems typified by high stocking densities and heavy feeding have been carried out in response to the rising output of white shrimp. However, this intensive farming approach has the potential to spread illness and reduce white shrimp productivity [3]. After China, India, and Vietnam, Indonesia is the fourth largest shrimp producer, accounting for around 4.6 percent of global shrimp production. The production of Pacific white shrimp requires the availability of high-quality larva in sufficient quantities and at the right time [4]. To increase the yield of vannamei shrimp culture, many biological agents have been used, one of which is the use of probiotic bacteria.

According to [5], probiotics are live microorganisms which if given in sufficient quantities will have a beneficial effect on the health of the host organism. *Bacillus* sp., *Photobacterium* sp., and *Lactobacillus* sp. are among the bacteria that contain probiotics [6]. Probiotic bacteria must meet certain criteria, such as being resistant to low pH and bile salts, as well as being able to thrive in the digestive system, create antimicrobial chemicals, adhere to intestinal cells (adhesion), be mass-produced, and remain stable and viable in the environment. long time in storage and in the field, as well as having a positive impact on the host [7,8,9]. The addition of probiotic bacteria to larval fish tanks (from egg to transformation) boosted survival, size uniformity, and growth rates, possibly due to changes in both the environment and the fish's microbial populations. Probiotics are one of the recognized alternatives to antibiotics, can suppress infections, and increase animal growth and health, as well as pond quality, and are therefore regarded an effective strategy for successful aquaculture. In people as well as in aquaculture, probiotic combinations are thought to be more efficient than single probiotic strains [10,11]. Probiotics are fed to aquatic animals to help them maintain their weight, size, and

nutrition [12]. The encapsulation approach can be utilized to improve and retain the character of these probiotic bacteria.

Encapsulation is a method of coating a material with a protective layer to protect it from environmental impacts. Microbes can be protected from negative environmental factors such as heat and chemicals by encapsulation in bacteria [13,14,15]. Non-toxicity, ease of application, and a low price are all requirements for the coating material, and the enclosed substance has comparably high cell viability and physiological qualities as before it was encapsulated. Encapsulation can increase the viability of probiotic bacteria compared to cells without encapsulation [15]. Based on the results of research by [15], it was shown that the storage of encapsulated *Lactobacillus* sp. for 2 weeks at 37°C produced a higher number of bacteria (17.3×10^7 CFU/mL at 10% skimmed milk concentration and 20.1×10^7 CFU/mL at 5% skimmed milk concentration) than before encapsulation (7.6×10^7 CFU/mL and 8.9×10^7 CFU/mL). In addition, the results of research by [16] showed that the resistance of bacteria *L. plantarum* sa28k and *L. plantarum* mar8 after spray drying with skimmed milk and gum arabic carriers was relatively good, which was around 89%. The number of bacteria before spray drying was 9.4 log CFU/g dry weight basis, after spray drying it decreased to 8.4 log CFU/g dry weight basis. The number of bacteria after being encapsulated by spray drying method for all encapsulated materials ranged from 10^7 - 10^9 CFU/g dry weight basis.

According to [17], one of the most extensively used and effective aquaculture tactics is biological control, which includes the use of probiotics. This is done with the understanding that using probiotic bacteria has several benefits, including that the organisms used are safer than using chemicals, that they do not accumulate in the food chain, that they can reduce repeated use in reproduction, that target organisms are rarely resistant to probiotic agents, and that they can be used for control. together. Many studies have been carried out on the use of probiotics in shrimp culture. The addition of probiotic bacteria to shrimp rearing containers can function as a complementary source of feed or contribute to the digestive system of the food and also suppress the population of pathogenic bacteria [17]. The results of the research by [17] showed

that the addition of fermented probiotics to the rearing media had a significant effect on the survival and production of vannamei shrimp. There was a tendency that the survival and production of vannamei shrimp was higher in the treatment using fermented products than the control without giving fermented probiotics. The results of trials and field observations conducted by [18] showed that the average survival of vannamei shrimp in the probiotic application treatment (79%-80%) tended to be higher than the control treatment (without probiotic application) which was 67%. In this study, a consortium of probiotics was tested which was encapsulated in the best carrier material (according to previous studies) on the performance of vannamei shrimp. The purpose of this study was to study the type of carrier material that can maintain the probiotic character and its effect on the performance of cultured vannamei shrimp.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were: vannamei shrimp fry, sea water, fresh water, probiotic culture (*Bacillus licheniformis*, *Bacillus subtilis*, *Lactobacillus brevis*, *Lactobacillus bulgaricus*, and *Lactobacillus curvatus*), encapsulate materials (maltodextrin, talc, and rice flour). This research was conducted at the Microbiology Laboratory and Greenhouse Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java-Indonesia.

2.2 Experiment Method

Experimental and descriptive research method was conducted on examining the effect of the probiotic consortium of *Bacillus* and *Lactobacillus* encapsulated in the best carrier material on the performance of vannamei shrimp. Measurements were carried out in the specific growth rate, survival, and feed conversion ratio of vannamei shrimp. The resulting data will be analyzed descriptively. The consortium used is derived from probiotics in the best carrier material that has been tested for characterization. The consortium formula used is:

A: Feed + Probiotics (*B. licheniformis* - talc + *B. subtilis* - talc)

B: Feed + Probiotics (*L. brevis* - rice flour + *L. bulgaricus* - maltodextrin + *L. curvatus* - maltodextrin)

C: Feed + Probiotics (*Bacillus* + *Lactobacillus*)
D: Feed (Control/without probiotics)

2.3 Procedures

Shrimp larvae of postlarvae stadia-12 (PL-12) aged 12 days were reared in an aquarium with a capacity of 10 liters and filled with 8 liters of seawater. The number of shrimp stocked was 80 per aquarium (10 shrimp/liter). The aquariums are aerated and arranged in large aquariums. Large aquariums that are used to accommodate small aquariums are filled with fresh water reaching the surface level of the rearing media and then given a heater, so that the temperature of the rearing media is stable at 29-31°C [19]. Shrimp larvae rearing was carried out for 14 days by measuring the performance of shrimp on the first and 14th days.

Probiotic bacteria were given when the shrimp larvae entered the postlarva-12 (PL-12) stage or at the beginning of rearing. The dose of feed given was 20% of the total weight of shrimp in each aquarium, with a dose of 3% probiotic and 97% commercial shrimp feed, and control in the form of commercial feed. The feed mixture is given directly to the larval rearing media 3 times a day. The feed used was TOP Yuh-Huei (Top Bucket Shrimp Flake) with 40% protein content, 3% fat, 3% fiber, and 17% ash content. The commercial feed was mixed with three different probiotic formulas for three treatments and one control treatment or feed without probiotics. The feed mixture must be able to sink at the time of spreading and have a durability of at least 2 hours in the water due to the nature of the shrimp that tend to live on the bottom of the water.

The specific growth rate of shrimp weight during the rearing period was calculated using a formula based on the research of [20], the specific growth rate of shrimp length was measured using the formula [19], Shrimp survival was calculated at the end of rearing using the formula [19] and the feed conversion ratio (FCR) during maintenance was calculated using the [21] formula.

3. RESULTS AND DISCUSSION

3.1 Specific Growth Rate

The specific growth rate of vannamei shrimp that was measured consisted of growth in weight and length of shrimp. The results of the calculation of the effect of probiotics on feed on the specific

growth rate of vannamei shrimp can be seen in Table 1.

Table 1. Specific growth rate of vannamei shrimp

Treatment	SGR (W) (g)	SGR (L) (mm)
A	0.48	8.77
B	0.42	7.65
C	0.62	9.81
D	0.48	7.66

Note: SGR (W): Specific growth rate of shrimp weight (%)

SGR (L): Specific growth rate of shrimp length (%)

Based on Table 1, it can be seen that the consortium treatment of *B. licheniformis* in talc, *B. subtilis* in talc, *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin and *L. curvatus* in maltodextrin resulted in the highest growth rate of vannamei shrimp weight of 0.618%. This indicates that the average weight of vannamei shrimp in this treatment experienced a higher increase than the other treatments. Meanwhile, the highest specific length growth rate of vannamei shrimp was also found in the same probiotic consortium treatment, which was 9.809%. This indicates that the average length of vannamei shrimp in this treatment experienced a higher increase than the other treatments.

Supplementation of probiotics can increase the specific growth rate of vannamei shrimp. The effect of probiotic bacteria on growth is thought to occur due to controlling the balance of microbes in the digestive tract, increasing the absorption of feed nutrients, and improving the nutritional value of feed [19]. The increase in growth rate is also thought to be due to the contribution of digestive enzymes by probiotic bacteria which can increase digestive activity. In the process of increasing digestive activity, probiotics have a mechanism to produce several exogenous enzymes for feed digestion such as amylase, protease, lipase, and cellulase [19,22]. In addition, the increase in growth can also be caused by an increase in feed nutrients (especially protein). Bacteria are one source of microbial protein so that the supplementation of bacteria in feed can increase feed protein [19].

According to [23], the use of probiotics in cultivation has been widely practiced in recent years. Probiotics have special enzymes that help in the breakdown of complex molecules into simple molecules that facilitate digestion and

absorption of nutrients in the shrimp digestive tract. High absorption of nutrients is expected to increase growth and production, thus supporting optimal cultivation business.

Meanwhile, according to [17], shrimp growth is influenced by heredity, sex, age, density, parasites, and disease, as well as the ability of shrimp to utilize food. Body weight gain is strongly influenced by feed consumption, because feed consumption determines the input of nutrients into the body which is then used for growth and other purposes. [17] suggested that proper feeding both quality and quantity can provide optimum growth for shrimp.

3.2 Survival Rate

Measurement of the survival of vannamei shrimp was carried out to determine the number of shrimps that lived until the end of the rearing. The number of initial shrimps kept as much as 80 individuals per aquarium. The results of the calculation of the effect of giving probiotics to feed on the survival of vannamei shrimp can be seen in Table 2.

Table 2. Survival of vannamei shrimp

Treatment	N ₀	N _t	SR (%)
A	80	65	81.67
B	80	45	55.83
C	80	68	85.42
D	80	67	82.92

Note: N₀: Number of shrimp at the beginning of rearing

N_t: The number of shrimp at the end of rearing

SR: Vannamei shrimp survival

Survival rate is the chance of an organism's life in a certain time [19]. The results showed that the highest survival rate of vannamei shrimp was found in the consortium treatment of bacteria *B. licheniformis* in talc, *B. subtilis* in talc, *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin and *L. curvatus* in maltodextrin of 85.42% with the number of shrimp that lived at the end of the rearing was 68 tails. However, the lowest survival was found in the consortium treatment of *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin, and *L. curvatus* in maltodextrin at 55.83% with only 45 shrimp surviving.

The loss of shrimp in large numbers was probably due to the lack of oxygen content of the water in the treated aquarium due to the increased turbidity of the water. This increase in

turbidity was caused by feeding extensively and the excretion of vannamei shrimp. This is in accordance with the statement of [24] which stated that the low dissolved oxygen content in shrimp rearing ponds can cause mass mortality of shrimp. When the oxygen content is reduced, shrimp can experience stress and cause death.

Ammonia content can also cause large numbers of shrimp that did not survive in maintenance. The amount of leftover feed that is not eaten and the results of shrimp excretion can increase the amount of ammonia in the water which if its content is above the threshold, it will be toxic and can poison vannamei shrimp. To reduce the ammonia content in water, biological agents can be used, one of which is *Nitrosomonas* bacteria. According to [25], the presence of nitrifying bacteria such as *Nitrosomonas* sp. and *Nitrobacter* sp. can oxidize toxic ammonia to nitrate which is less toxic to shrimp.

Water quality is very important to consider in the maintenance and cultivation of vannamei shrimp. This is in accordance with the statement of [26] which stated regarding the factors that most influence the survival rate of vannamei shrimp larvae are water quality in the rearing media and feed quality. Good water quality in maintenance media will support metabolic processes in physiological processes.

Apart from the consortium treatment of *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin, and *L. curvatus* in maltodextrin, other treatments showed a fairly high survival value of vannamei shrimp with a value above 80%. This indicates that both the treatment with the addition of probiotics and the control can increase the

viability of vannamei shrimp. The high survival rate is thought to be because the feed given has high protein and can be utilized properly, so that environmental factors are maintained in the rearing media that can support shrimp survival and reduce stress conditions that allow death during rearing [26].

3.3 Feed Conversion Ratio

Feed conversion ratio (FCR) is an indicator to determine the effectiveness of feed and is one of the parameters used to describe the amount of feed that can be utilized by cultured organisms. FCR is the opposite of feed efficiency, meaning that the higher the FCR, the lower the feed efficiency obtained and vice versa [19]. The results of the calculation of the vannamei shrimp feed conversion ratio can be seen in Table 3.

Based on Table 3, it can be seen that the best vannamei shrimp feed conversion ratio was found in the consortium treatment of *B. licheniformis* in talc, *B. subtilis* in talc, *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin and *L. curvatus* in maltodextrin with a value of 1.08. This shows that the provision of the probiotic consortium in the feed is able to increase the utilization of feed more effectively, so that the use of feed is more efficient in providing a good response to the value of the feed conversion ratio. Based on the data obtained, it can be seen that the probiotics consisting of a consortium of *Lactobacillus* and *Bacillus* produced significantly higher FCR compared to the use of single probiotic bacteria. It is suspected that there is a symbiotic effect between bacteria which

Table 3. Vannamei shrimp feed conversion ratio

Treatment	Repetition	FCR	Mean
Feed + Probiotics (<i>B. licheniformis</i> + <i>B. subtilis</i>)	1	1.59	1.87
	2	3.16	
	3	0.86	
Feed + Probiotics (<i>L. brevis</i> + <i>L. bulgaricus</i> + <i>L. curvatus</i>)	1	2.05	1.82
	2	2.34	
	3	1.06	
Feed + Probiotics (<i>Bacillus</i> + <i>Lactobacillus</i>)	1	1.24	1.08
	2	1.09	
	3	0.89	
Feed (Control)	1	1.09	1.35
	2	1.35	
	3	1.58	

Notes: FCR: Feed Conversion Ratio

produces more complete enzymes and increases the FCR of shrimp. Multiple strains of probiotics result in additive or even synergistic effects, or reduced effects due to mutual inhibition [10]. Further [10] reports diets incorporating individual or combined species of the probiotics, *B. subtilis* E20 and *L. plantarum* 7–40, were fed to the mud crab, *Scylla paramamosian* for 28 days, and results indicated that a reduced efficiency of combined species of probiotics was found due to an antagonistic effect of growth between two probiotics. The same results were obtained in [22] study which calculated the value of the feed conversion ratio in milkfish with the addition of the probiotic bacteria *Carnobacterium* sp.

According to [27], feed efficiency can be achieved if the rearing of fish/shrimp pays attention to feeding management, because the feed consumed by cultured organisms will in turn be used to grow. Therefore, feed that is less than the minimum requirement of cultivated organisms to maintain body weight will result in weight loss due to food reserves in the body being used to meet the energy needs of their activities. [27] suggested that proper feeding both quality and quantity can provide optimum growth for shrimp. Feeding in excessive amounts will increase production costs and waste and cause excess feed residue to reduce water quality so that it affects the growth and survival of shrimp.

4. CONCLUSION

Based on the results of the study, it can be concluded that supplementation of a consortium of encapsulated bacteria *B. licheniformis* in talc, *B. subtilis* in talc, *L. brevis* in rice flour, *L. bulgaricus* in maltodextrin and *L. curvatus* in maltodextrin in commercial feed can increase the specific growth rate, survival, and feed conversion ratio of vannamei shrimp. The results showed that the fifth consortium of Bacillus and Lactobacillus bacteria could increase the growth rate of weight and length of vannamei shrimp (0.62% and 9.81%), survival rate (85.42%) and feed conversion ratio (1.08).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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