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Research Article
Effectiveness of Immersion with Probiotic in Improving the Health of Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Background and Objective: Probiotic is a live microorganism, capable of improving health if given the right and sufficient amount. This research is aimed at analyzing the impact of probiotic bacteria immersion, isolated from giant river prawn and giant tiger prawns, towards the health of Nile tilapia. It was conducted from February-June, 2017. **Materials and Methods:** For this experiment, treatment was used a completely randomized design (CRD)with 5 treatment phases and 3 replicates. The Nile tilapia was immersed into probiotic bacteria as our treatment for 5 min in every 10 days during 30 days of culture. Subsequently, intracellular technique, as much as 0.1 mL/fish infected the fish, with 10^5 CFU mL^{-1} density of *Streptococcus iniae*. **Results:** Both isolated sources of probiotic immersion have a positive impact on the health of tested tilapia. The P3 treatment (immersion with UWH9 strain) is the best treatment as the total of erythrocytes reached 174.67×10^4 cells mm^{-3}, with 7.47 g dL^{-1} hemoglobin, 33.67%, hematocrit, 80.00×10^3 cells mm^{-1} leucocytes and 80% survival rate. **Conclusion:** Immersion with probiotic bacteria isolated from giant freshwater and giant tiger prawns digestive system could enhance the health quality of Nile tilapia. The type of probiotic P3 isolates (UWH9) as the best isolate, isolated from digestive track of giant tiger prawn (*Penaeus monodon*).

Key words: Probiotic, erythrocytes, infectious, Nile tilapia, immunity

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Nile tilapia (*Oreochromis niloticus* sp.) is one of fish that is commonly cultured by fish farmers in Indonesia. However, this species is prone to be attacked by *Streptococcus iniae* and a case of massive death once happened in Sumatera Island, Indonesia. Now-a-days, there has been an increasing amount of fish culture in Indonesia. At the same time, the development of people activities nearby the fish culture has also increased. This has inevitably brought about some impacts on the fish culture. The situation is aggravated by the emergence of upwelling, which at that time has catalyzed a massive death to fish in Sumatera[1]. In addition, it is found that freshwater fish, with the exception of Pangasius and Nile tilapia, are good sources of n-3 fatty acids, especially EPA and DHA[2]. These fatty acids in freshwater fish are on approximately the same or higher level in relation to some of the marine fish examined. Pangasius and Nile tilapia are characterized by the highest content of saturated fatty acids, where salmon, flounder and rainbow trout have the highest values of monounsaturated fatty acids.

An attempt to improve the stamina and immunity of the fish without side effects has currently been developed; however, a more probiotic study is still needed. The provision of probiotic on fish will be beneficial to enhance the cellular non-specific immunity system. It can increase the number of kidney macrophage and the activity of phagocytosis. Besides that, it might play an important role in suppressing the growth of pathogenic microbial populations. Probiotic bacteria and including lactic acid bacteria have an ability to produce several antimicrobial compounds such as lactic acid, di-acetyl, hydrogen peroxide, carbon dioxide and bacteriocins[3].

The probiotic of *Bacillus* sp., has extensively been applied for the biotechnology interest, including the contents of various enzymes and its amino acid. It is also used to produce antibiotics to control the pathogens[4]. This type of probiotic also has been implemented on the media of fish culture within an aquarium with 10^4 CFU mL^{-1} dosages, which proves to inhibit the growth of *Aeromonas hydrophila* on Dumbo catfish (*Clarias gariepinus*)[5]. This research used probiotic that had been isolated from giant freshwater and giant tiger prawns digestion from Riau[6]. The use of this probiotic of *Bacillus* sp., that isolated from these two species of prawns is not much used yet. Therefore, the aim of this study is to determine the effect of *Bacillus* sp., probiotic immersion isolated from giant freshwater and giant tiger prawns towards the health of Nile tilapia (*Oreochromis niloticus* sp.), as a form of its protection to *Streptococcus iniae* bacteria.

RESEARCH METHODS

The study was conducted from February-June, 2017 in Marine Microbiology lab and in the Lab of Parasites and Fish Diseases, at Faculty of Fisheries and Marine Sciences, Riau University. This study used a completely randomized design (CRD) with 5 treatments and 3 replicates.

This experiment consisted of two steps. The first step was the immersion of fish culture into the container of probiotic bacteria regularly, 5 min every 10 days during 30 days of culture and the density of the probiotic bacteria was 10^5 CFU mL^{-1}. It was done to let the fish to get probiotic *Bacillus* sp., that had been isolated from giant freshwater prawn (*Macrobranchium rossenbergii* DE MAN) and giant tiger prawn (*Penaeus monodon*). During the culture, the fish were given F-999 commercial fodder for 3 times a day.

There are 5 types of treatment in this experiment, namely, (1) PO (control without probiotic), (2) P1 (the immersion of fish into probiotic isolate taken from giant freshwater prawn/GU4), (3) P2 (the immersion of fish into mixing probiotic isolate from giant freshwater/UG1+UG2+UG3+UG4+UG5), (4) P3 (the immersion of fish into probiotic isolate taken from giant tiger prawn/UWH9, (5) The immersion of fish into mixing probiotic isolate/UWH1+UWH2+UWH8+UWH9+UWH10).

The blood of the fish was drawn after 30 days of culture and then *Streptococcus iniae*, pathogenic bacteria, was injected into the tissue of fish. The density of the pathogenic bacteria injected was as much as 10^5 CFU/tested fish. The blood of the fish was redrawn after 14 days of culture to prove that there were effects of immersion into probiotic bacteria against the immunity of the fish on the pathogenic bacteria injected. The indicators used in this experiment were the total number of erythrocytes, the level of hemoglobin and hematocrit, total leucocytes and survival rate.

The bacteria samples of *S. iniae* used were taken from the Laboratory of Parasites and Fish Diseases, Laboratory of Fish Quarantine Station ClassI, Sultan Syarif Kasim II, Pekanbaru. The 150 samples of Nile tilapia (10 fish in each aquarium) were gained from Fish Hatchery Hall (BBI) Sipungguk, Kampar Regency.

The probiotic isolate used originates from giant freshwater prawn and giant tiger prawn that were sequenced with 16S rDNA. They were afterward recultivated by nutrient agar (NA) media and Nutrient Broth (NB). As for the anesthesia, clove oil was used. Last, regarding the blood check, substances like Na-Sitrat (anticoagulant), Turk solution, Hayem solution, HCl 0.1%, Aquades, vortex candle and F-999 commercial pellet were used.

The tools utilized were 40×30×30 cm aquarium complete with its aeration equipment, analytical scales, a test tube, a petri disc, an autoclave, an incubator, a syringe, a binocular microscope, a haemocytometer, an object and a cover glass, a DO-meter, a pH meter, a spectrophotometer and many others.

Fifteen aquariums were used to culture the fish tested during the study. It was 40×30×30 cm in size and completed with aeration equipment each. Before being used, all aquariums were cleaned up to get rid of pathogenic microorganism by using $KMnO_4$ solution for 24 h. Then the aquariums were rinsed and dried. After that, they were filled with 25 cm of water (30 L) and aerated. Finally, ten Nile tilapias (8-12 cm in size) were put into each aquarium.

Probiotic preparation: Probiotic originating from a giant freshwater prawn orgiant tiger prawn was re-grown on the NA media and incubated at 27-28°C temperature for 24 h. It was then harvested by means of taking the probiotic growing on the agar NA for 1 ose and put to the NB (nutrient broth) media with 50 mL (as starter bacteria) volume. Lastly, a 24 h incubation was conducted.

The starter bacteria flourishing on the NB media was subsequently mixed to the NB media for about 250 mL, so that the probiotic volume reached 300 mL. Next, in order to ensure and obtain 10^5 CFU mL^{-1} probiotic bacteria density, a gauge using spectrophotometer was carried out.

Further, the mixed probiotic from the digestive track of giant freshwater prawn (UG1+UG2+UG3+UG4+UG5) and giant tiger prawn (UWH1+UWH2+UWH8+UWH9+UWH10) was organized. Each isolate with the same amount was taken and brought into the Erlenmeyer flask (2 elenmeyer flasks were utilized) for every mixed probiotic of giant freshwater and giant tiger prawns.

Handling of tested fish: The tested fish were cultivated 30 days during the study and immersed for 5 min into probiotic isolates every 10 days. The temperature of the fish cultured environment was kept 28°C by using the heater. Siphoning was done every day to scour the remaining fodder and feces by reducing the water volume to 50%. The immersion procedure was accomplished by taking the fish out of the aquarium and moving them to the immersion container. After being immersed, the fish were again transferred into the aquarium. At the last stage of the pisciculture, there was a blood sampling and an infection taken by using *S. Iniae* bacteria. At the post-infection activity, the fish blood test was done all over again.

The observed parameters during the research are as follows.

Total erythrocytes: The total erythrocytes measurement was conducted by absorbing the blood sample using 0.5 scaled pipette and followed by absorbing the Hayem solution until 101 scales. After that, it was homogenized by way of shaking it to form an 8 figure. The first drop was discarded and the following drops were put into the hemocytometer and covered with cover glass. The measurement was done within 5 small boxes of the hemocytometer. The total was calculated with the following formulation[7]:

$$\text{Total erythrocytes} = \text{Total counted erythrocytes cell} \times 10^4 \text{ cell mm}^{-3}$$

Level of hemoglobin: Total hemoglobin was gauged by filling the salinometer with HCl 0.1 N solution until it reached 0 (the lowest scale line on salinometer). Hereafter, the tube was positioned between 2 tubes with standard color. For 0.02 mL of fish blood was then taken by using a microtube with sahli pipette. The blood was put into salinometer and stilled for 3 min after the tip of the pipette was firstly sterilized. Moreover, aquades was added by using a drip pipette bit by bit while being stirred with a glass stirrer until the color is exactly similar to the standard color. The level of hemoglobin is stated in g dL^{-1} [7].

Level of hematocrit: The blood sample was put into the hematocrit capillary tube until approximately four-fifths of the tube. Then, the tip (red sign) was blocked with critoseal and centrifuge for 15 min at 3500 rpm speed. After that, the percentage of the hematocrit was measured. The hematocrit level is presented as percentage of blood cell volume[7].

Total leucocytes: The measurement of total leucocytes was carried out by absorbing the blood sample with 0.5 scale pipette (special pipette for leucocytes measurement). This was followed by absorbing the Turk solution to 11 scales and then homogenized by shaking it to form an 8 figure. The first drop of blood was thrown away and the next drops were put into the hemocytometer and covered with cover glass[7]. This gauge was done in 4 hemocytometer big boxes and counted by using the formulation below:

$$\text{Total leucocytes} = \text{Total counted leucocytes cell} \times 50 \text{ cell mm}^{-3}$$

Survival rate: In order to determine the survival rate, the formulation of Zonneveld *et al.*[8] was used:

SR = Survival rate (%)
Nt = Fish population at the end of culture (quantity)
No = Fish population at the beginning of culture (quantity)

Water quality: The water quality of the cultured fish was only measured twice during the experiment. It was at the beginning and at the end of the experiment. These water parameters consisted of temperature, pH and dissolved oxygen (DO).

Statistical analysis: All statistical comparisons used one-way analysis of Variance (ANOVA)[9] and Duncan test. The differences were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Total erythrocytes: The results showed that the number of erythrocytes in the blood of fish increased after treating in regular immersion into probiotic *Bacillus* isolates. The highest was found at P3 treatment ($182.67\pm7.637\times10^4$ cells mm^{-3}) while the lowest was at P0 without treatment ($152.33\pm5.131\times104$ cells mm^{-3}) (Fig. 1). This indicates that the probiotic immersion can increase the number of erythrocytes in the blood of Nile tilapia. Furthermore, all figures were lower after the treated fish were injected with infectious *S. iniae* bacteria. However, the values are still higher compared to control treatment (P0).

After the infection with *S. Iniae*, the number of erythrocytes within Nile tilapia blood decreased (Fig. 1). Although there was a reduction in the number of erythrocytes, the *Bacillus* probiotic provision proves to be capable of preserving the number of erythrocytes in the blood of Nile tilapia as an influence of *S. Iniae* infection. The decline of the erythrocytes has assumingly occurred for the extracellular product generated by the *S. Iniae,* that is hemolysin, an extracellular enzyme having the ability to lyse erythrocytes by producing a toxin in a form of hemolicin[10].

It is likely that the erythrocytes total reduction after the infection happened because of the entrance of phagocytosis bacteria. The process needs oxygen resulting in a decrease in erythrocytes. The bacteria entering the body would undergo phagocytosis process where the phagocyte cells will recognize and digest the requiring oxygen bacteria particles so that erythrocytes decline is happening[11].

Based on the statistical test (ANOVA), it shows that the erythrocytes amount of Nile tilapia given probiotic treatment, which is isolated from the digestive track of giant freshwater prawn and giant tiger prawn, has an effect on the number of erythrocytes after infection with *S.iniae bacteria*, observed on the P3 treatment compared to the control of P0 ($p<0.01$). It designates that the *Bacillus* sp., probiotic provision indeed has a tangible impact towards the change of the erythrocytes

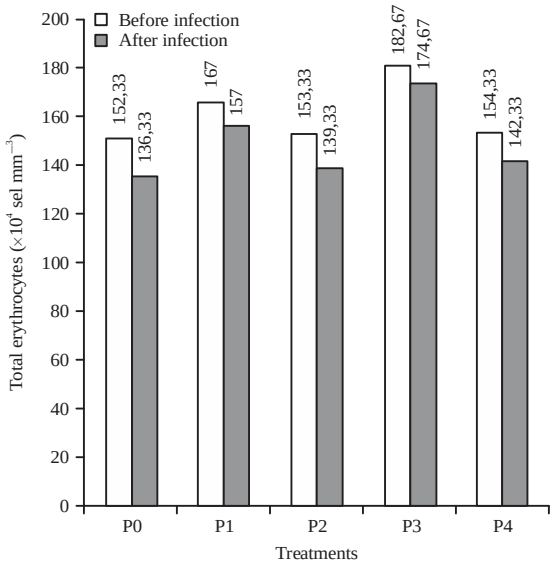

Fig. 1: Number of erythrocytes for each treatment before and after infection by *S. iniae*

P_0: Control treatment(without probiotic and *S. iniae* immersion and infection), P_1: Immersed with *Bacillus* sp., probiotic, UG4 isolate and *S. Iniae* infection, P_2: Immersed with *Bacillus* sp., probiotic, collection of some isolates (UG1+UG2+UG3+UG4+UG5) and *S. Iniae* infection, P3: Immersed with *Bacillus* sp., probiotic, UWH9 isolate and *S. Iniae* infection, P4: Immersed with *Bacillus* sp probiotic, collection of some isolates (UWH1+UWH2+UWH8+UWH9+UWH10) and *S. Iniae* infection

number to Nile tilapia. Specifically, the number of erythrocytes of Nile tilapia provides crucial information as to the physiology condition and demonstrates its health status[12].

Level of hemoglobin: In terms of hemoglobin levels, the results showed the same pattern as erythrocytes. The level of the hemoglobin in the blood of fish after being treated with probiotic immersion was higher than P0 without treatment. The same pattern of the blood level was also seen after the tested fish injected with infectious *S. Iniae*. These results are identical with a research project conducted by Salasia *et al.*[13] where the level of hemoglobin of Nile tilapia ranged between 5.05-8.33 g dL^{-1}. This condition indicates that the *Bacillus* sp., probiotic provision can increase the number of hemoglobin in the blood contrasted to the control treatment of P0.

Once the infection finished, there was a decrease in the hemoglobin level in the blood of Nile tilapia (Fig. 2). Phagocytic activity seems to be the main cause of the decline of hemoglobin for its activity requires more oxygen in resisting pathogenic bacteria[14]. It was mentioned that several *Streptococcus* sp., yield hemolysin as a result of extracellular protein secretion of lipid membrane possessing the ability to lyse hemolysis, reducing the amount of hemoglobin and red blood cells.

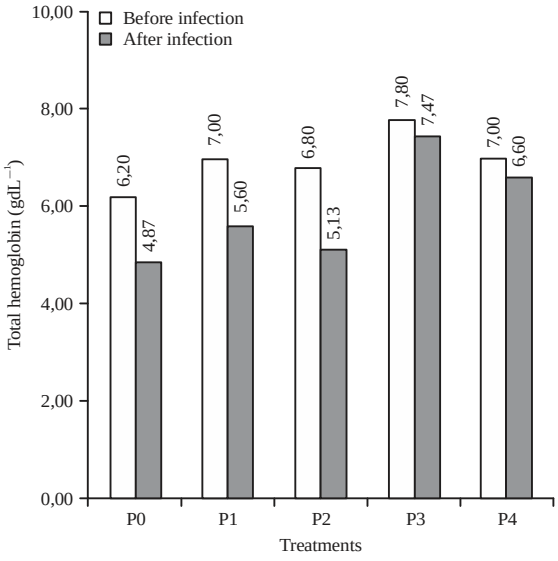

Fig. 2: Hemoglobin level in the blood of Nile tilapia (*O.niloticus*) at each treatment before and after injected with *S. Iniae*

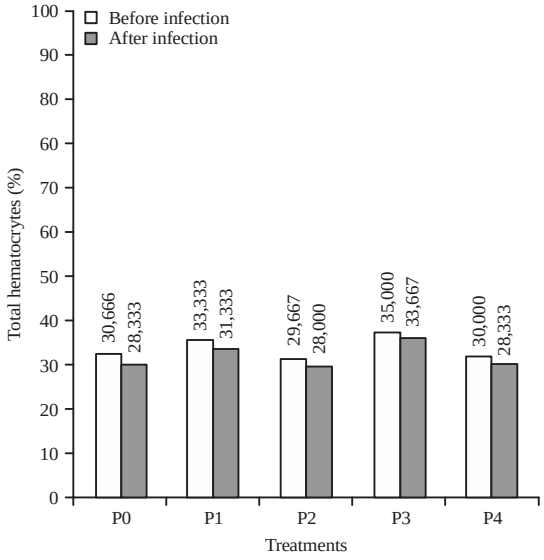

Fig. 3: Percentage of hematocrit level in the blood of Nile tilapia (*O. niloticus*) for each treatment, both before and after infected with *S. Iniae*

On the basis of a statistical test (ANOVA), it describes a significant distinctive influence ($p<0.05$) compared to the control treatment (P0) in terms of the level of hemoglobin of Nile tilapia given probiotic treatment isolated from the alimentary canal of giant freshwater prawn and giant tiger prawn after the infection with *S. iniae* bacteria. The low level of hemoglobin denotes that the *S. iniae*-infected Nile tilapia undergoes an erythrocytes disorder. The existence of *Streptococcus* sp., toxin affects the hemoglobin stability. The possibility of kidney infection engenders a low red blood cells production, causing anemia and a limited amount of hemoglobin to the fish[15]. Saputra *et al.*[16] add that the quantity of hemoglobin is linear with the number of erythrocytes. The higher the total of hemoglobin, the greater the number of erythrocytes.

Level of hematocrit: Based on hematocrit, the percentage of its number is steadily increased after treatments (P1 and P3) and a little bit decreased at P2 and P4. The same pattern was also identical with the treatment after *S. Iniae* infection (Fig. 3).

The previous study stated that the normal level of the hematocrit of Nile tilapia is around 27.3-37.8%[10]. This signifies that the probiotic *Bacillus* provision can improve the capacity of hematocrit in Nile tilapia blood. Nevertheless, hematocrit decline occurred after the infection with *S. Iniae* bacteria. This hematocrit intensity drop depicts a defect on the red blood cells due to bacteria infection[17].

Based on the statistical test (ANOVA), the control (P0) has no considerable difference with the P1, P2, P3 and P4 at the post-infection by using *S. iniae* bacteria ($p>0.05$). The total of the hematocrit of Nile tilapia after an infection with the *S. iniae* bacteria is still normal. The fish are able to maintain the hematocrit amount in the blood because an antibacterial substance within the probiotic is presumably existing. As stated by Sumardi *et al.*[18]. *Bacillus* sp. bacteria generate such immunity and antimicrobial as bacteriocin. The whole bacteriocin within the *Bacillus* bacteria may possibly control the pathogenic bacteria by inhibiting its growth and attacking the bacteria cellulose. Bacteriocin will hence give resistance to each pathogenic bacterium entering the body.

Total leucocytes: The result of observation on total leucocytes in the blood of Nile tilapia during treatment with probiotic shows the trends for an increase in the number of total leucocytes (Fig. 4). These figures are rather similar to a study conducted by Moyle and Cech[19], declaring that the number of leucocytes of Nile tilapia is generally between $20\text{-}150\times10^3$ cell mm^{-3}.

However, the total number of leucocytes at all treatments was lower than control after *S. Iniae* infection (Fig. 4). The leucocytes increase is caused by the appearance of and an infection initiated by *S. Iniae* bacteria. In addition, Matofani *et al.*[20] stated that the increase of the leucocytes is related to cellular defense.

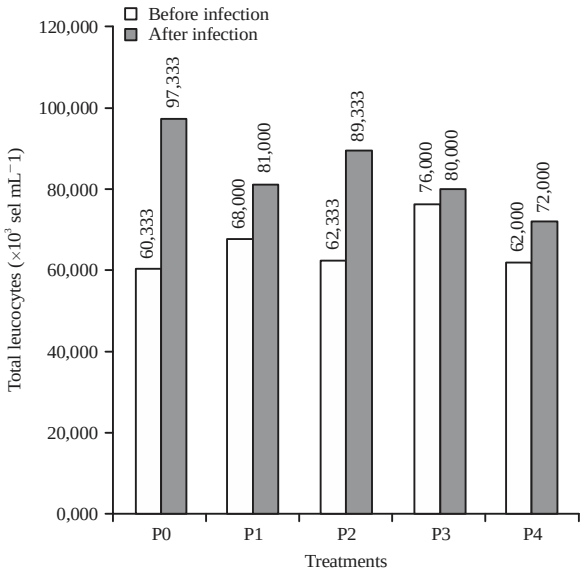

Fig. 4: Total leucocytes in the blood of Nile tilapia (*O. niloticus*) at each treatment before and after *S. Iniae* infection

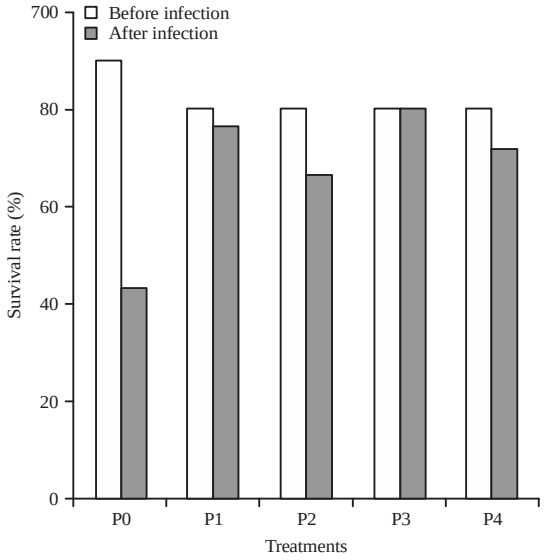

Fig. 5: Survival rate of Nile tilapia (*O. niloticus*) for each treatment in both before and after *S. iniae* infection

A statistical test (ANOVA) points out that the total of leucocytes of Nile tilapia given *Bacillus* probiotic treatment, which is isolated from the alimentary canal of both giant freshwater prawn and giant tiger prawn after an infection with *S.iniae* bacteria (P1, P2, P3 and P4), is truly dissimilar to the control (P0) ($p<0.01$). Obviously, it designates that the *Bacillus* probiotic provision could suppress the *S. iniae* infection. Todar[21]; Torkar and Martijasic[22] explain that apart from yielding bacteriocin antimicrobial, *Bacillus* also has other antimicrobial chemical compounds, such as bacitracin, pumulin, gramicidin, laterosporin and tyrocidine, which effectively oppose the Gram-positive bacteria, as well as colistin and polymyxin, functioned to well-resist the Gram-negative bacteria.

Immunity can be improved by affording probiotic in three different ways[23]. First, improving the macrophage activity that can be seen from the microorganism increase, undergoing phagocytosis or carbon particles. Second, improving a systematic antibody production like immunoglobulin and interferon. Lastly, improving local antibody on the mucus surface such as to the intestinal wall.

When something or an object goes into the body, leucocytes will perform a phagocytosis activity and form an immunity system and antibody, affecting the rise of leucocytes intensity in the blood. The action is implicitly a clue of resistance to the pathogenic bacteria. Matofani *et al.*[20] declare that the number of leucocytes is multiplied, occurring as a result of the cellular resistance system from within the body. In addition, probiotic also contains bacteriocin having its own mechanism in hampering the pathogenic bacteria growth and even causing death to the bacterial cell, sensitive to bacteriocin[24]. Bacteriocins from probiotic bacteria produced from the LAB isolates may be useful as a food bio-preservative for controlling microbial deterioration, enhancing the hygienic quality and extending the self-life of fish and seafood products[25].

Survival rate: The observation result on the survival rate of Nile tilapia after 30 days of culture with probiotic immersion shows that their survival rate was high and the highest one was found at control (P0). It is suspected that Nile tilapia undergo an adaptation process at the time probiotic was given (Fig. 5).

Figure 5 describes that Nile tilapia has a well survival rate with >75% post-infection. In addition, the treatment with probiotic immersion has high survival rate compared with control without treatment. Tabasco *et al.*[26] assert that the probiotic use can develop the survival rate and immunity of fish towards pathogen infection.

Grounded in the statistical analysis (ANOVA) after infection, the survival rate of Nile tilapia having probiotic isolated from alimentary canal of giant freshwater prawn and giant tiger prawn was found. The P0 treatment significantly affects the following P1, P2, P3 and P4 ($p<0.01$). In other words, the probiotic provision implemented by immersion is powerful in a well-raising survival rate of Nile tilapia. Silva *et al.*[27] argue that the usage of *Bacillus* sp., type of probiotic is very much proven to improve and preserve the survival rate of cultured aquatic organisms. Furthermore, Sorokulova *et al.*[4] also affirm the fact that *Bacillus* type of

Table 1: Water quality of media culture of Nile tilapia (*O. niloticus*) during the study

	Treatments					
Parameters	P0	P1	P2	P3	P4	Kordi[30] (2010)
Temperature (°C)	27.8	28	27.8	28	28	25-33
DO (mg L^{-1})	3.9-4.3	3.9-4.6	4.0-4.2	3.9-4.6	4.0-4.2	>3
pH	6.8-7.0	6.4-7.1	6.2-7.0	6.4-7.1	6.2-7.0	6.0-8.5

probiotic has greatly been applied, especially to biotechnology importance, including enzymes, amino acid and antibiotics to control pathogens. Probiotic in aquatic culture plays a role to sustain the living performance and enhance immunity system by changing the bacteria group[28]. Jeong *et al.*[29] maintain that using the vaccine MVACINIAE has a protective effect on the wild-type of *S. iniae* (SI-36) in olive flounders on Jeju and immunity against *S. iniae* lasted for at least 6 months. However, the application of the vaccine to control *S. iniae* in the olive flounder on Jeju should be done based on the prevalence of the disease.

Water quality of treatment media: The measurement result of water quality during the research shows the temperature on the treatment. In P0, the temperature was 27.8°C and P1's temperature was 28°C. Further, P2 had the same temperature as P0 and P3 and P4 temperature were also similar to P1. The value of dissolved oxygen (DO) on P0 was 3.9-4.3 mg L^{-1}. As for P1, P2, P3 and P4, the dissolved oxygen was, respectively 3.9-4.6, 4.0-4.2, 3.9-4.6 and 4.0-4.2 mg L^{-1}. Regarding the pH, its proportion in P0 was 6.8-7.0, P1 as 6.4-7.1, P2 as 6.2-7.0, P3 as 6.4-7.1 and P4 as 6.2-7.0. The enumeration is identical to Kordi[30] stating that the temperature approximation friendly to the life of Nile tilapia is 25-33°C, with >3 mg L^{-1} dissolved oxygen and water pH is going between 6.0-8.5.

The water temperature on each treatment is just about 27-28°C, still also deemed as a safe level for the Nile tilapia environment. This is in line with Warasto *et al.*[31], asserting that the suitable temperature for the growth of Nile tilapia is 25-30°C.

Temperature is a fundamental parameter for waters organisms because of its effect on metabolism activity of the organisms. The temperature is influential to fish stability, continuity and growth. In general, the growth pace is parallel with the temperature increase. This can make the fish stressful, or even trigger death to the fish if the temperature dramatically increases[30]. The specific information can be seen in the Table 1.

The water pH during the treatment is going around 6.0-7.2. Thus, it can be inferred that the estimated pH measured throughout the treatment is still acceptable for the survival of Nile tilapia. The dissolved oxygen in the treatment is 3.9-4.6 ppm. Alfia *et al.*[32] declares that the amount of dissolved oxygen in waters which is safe for Nile tilapia is >3.5 ppm. Hence, the dissolved oxygen discovered during the treatment reveals the right amount of the growth and life of Nile tilapia. Other than that, the influence of the environment also plays a major role in maintaining Nile tilapia's health. According to Abdelhamid *et al.*[33] these drastic effects related to the presence of some pollutants from agricultural and urban drainages whether in the rearing water or in the feeding dried sewage sludge (DSS) can negatively affect fish health, production and quality, as well as could be inter the food chain and threaten human health.

CONCLUSION AND RECOMMENDATION

The probiotic provision, either isolated from giant freshwater prawn (*M. rosenbergii* de MAN) or giant tiger prawn (*P. monodon*), shows a positive indication in affecting and improving the health of Nile tilapia (*O. niloticus*). The research reveals that *Bacillus* sp., type of probiotic P3 isolates (UWH9)is the best isolate which is isolated from alimentary canal of giant tiger prawn (*P. monodon*). The blood parameters after infection with *S. iniae* bacteria demonstrate findings as the following: 174.67×10^4 cell mm^{-3} total of erythrocytes, 6.65 g dL^{-1} hemoglobin, 33,67% hematocrit, 80.00×10^3 cell mm^{-3} leucocytes and 80% survival rate. The water quality measurement result during the research was around 27-28°C, 4.0-4.6 ppm dissolved oxygen and 6.2-7.1 pH.

A further research project needs to be accomplished to scrutinize the histopathology description of Nile tilapia (*O. niloticus*) after being treated with probiotic immersion, isolated from alimentary canal of giant freshwater prawn (*M. rosenbergii* de MAN) and giant tiger prawn (*P. monodon*), infected by *S. iniae* bacteria.

SIGNIFICANCE STATEMENT

This is the first study analyzing the probiotic isolates taken from giant freshwater prawn and giant tiger prawn. The outcomes of this study will be beneficial for fish culture in the future.

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