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The Role of Probiotic Bacteria on Controlling Vibriosis in Tiger Grouper Fry (*Epinephelus foscoguttatus*)

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Abstract: Tiger grouper is one of marine fish commodities well-loved by the community and have high economic value. Increased production of grouper has stimulated disease infection by virus, bacteria, or parasitic organism in aquaculture environment this experiment was conducted to evaluate the efficacy of probiotic bacteria in controlling vibriosis disease in tiger grouper fry. The probiotics feed were isolates K7, K8, T36 and T41. Administration of probiotics was performed for 28 days and on day 29 challenge test was performed using immersion method in pathogen *Vibrio* solution at concentration of 10^6 CFUmL^{-1}. Population bacteria in the liver were observed evey six hours for 48 hours. The results showed that the four isolates of probiotic bacteria can reduce pathogen *Vibrio* populations in the tiger grouper on liver. The survival rates before challenge test in the probiotic treatments and control were from 65.00 to 88.33% and 71.67%, respectively. After challenge test, survival rates in the probiotic treatment and positive control were from 51.67 to 78.33% and 48.33%, respectively. Growth body weight and length in the probiotic treatments were from 7.28 to 8.42% and 2.94 to 3.28%, respectively, while in control 7.07% and 2.92%, respectively. The administration of probiotics T36 provided the best results compared to other isolates. Addition of probiotics can suppress population of *Vibrio* pathogen and incresed the surviral rate of tiger grauoper fry. The use of probiotics can increase the production of tiger grouper.

Key words: Probiotics · Contolling · Tiger grouper · Fry · Growth

INTRODUCTION

Tiger grouper is one of marine fish commodities well-loved by the community and have high economic value. Increased production of grouper has stimulated disease infection by virus, bacteria, or parasitic organism in aquaculture environment. Vibriosis is a bacterial disease that often attacks the grouper culture. *Vibrio alginolyticus* and *V. carchariae* known to an agent that causes the disease vibriosis in aquaculture of grouper [1]. It has been reported that *V. alginolyticus* is an agent associated with vibriosis illness and has caused mass mortality in the cultivation of large yellow croakers and caused economic losses [2]. Some pathogens such as *V. anguillarum, V. parahaemolyticus, V. alginolyticus, V. vulnificus, V. harveyi and V. carchariae* are known to cause severe haemorrhagic septicemia and gastroenteritis syndrome [1].

One way that can be used to control diseases in aquaculture is by strengthening the immune system of fish by using probiotics [1]. Various mechanisms have been demonstrated from the use of probiotics includding inhibition of pathogens through the production of inhibitory compounds, competition for sticking place, competition for nutrients, improvement of water quality [3, 4]. Moreover, probiotics also inhibit the

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expression of virulence genes or disrupt qorum sensing mechanism, as a source of micro-and macronutrients and increase the contribution of enzymatic digestion system [5].

Several studies have shown that microorganisms have a beneficial effect in the animal digestive process waters contributing nutrients, a source of food supplements, a source of vitamins and essential amino acids [6], affecting the activity of digestive [7-10].

Probiotics to be used in this study were isolates K7, K8, T36 and T41 as the result of the selection in the previous study [11]. These isolates have been tested and have shown ability to inhibit the growth of pathogenic *Vibrio,* to produce digestive enzymes and non-pathogenic to tiger grouper. While the pathogen Vibrio, *Vibrio* V6 used was identified as *V. parahaemolyticus* using marker of resistant rifampicin (V6 Rf^R). This study aimed to evaluate the efficacy of probiotic bacteria in controlling the disease vibriosis in fry tiger grouper.

MATERIAL AND METHODS

Tiger grouper fry (*E. foscoguttatus*) was obtained from seeding activity in Brackish Water Aquacultur Center Situbondo. Fish with average weigth of 0.235±0.03 g and length of 2.00±0.18 cm was maintained with density of 20 fry/container at volume 60 L. To reduce of stress due to the influence from the outside, the counteriner was covered by blue plastic. Before used, all equipements and water were sterilized with chlorine at concentration 150 mgL^{-1} for 24 hours and then neutralized with sodium thiosulfate 75 mgL^{-1} and aerated strongly. Water exchange was performed twice a day.

Feed preparation includes the step of feeding, the probiotic bacterial cultures and mixing feed. Fist, cultured probiotic bacteria in the liquid SWC (Sea Water Complete). Harvesting is done after reaching exponential phase (16-18 hours). Bacteria populations were measured using a spectrophotometer at 620 nm. After that, the probiotic culture transferred tube 50 mL and then centrifuged for 15 minutes at 4.000 rpm to separate solids bacterial cell supernatant. Probiotic pellet obtained was then washed with a solution of PBS (Phosphate Buffer Saline). Pellets are probiotic bacteria and then mixed into the feed as much as 1% [12], the mixing is done by adding egg yolk which serves as adhesive as much as 2% by spraying evenly using a syringe and dried aired.

Feed used was in the form of commercial diets with protein content of 48%. In the first ten days, feed used was feed powder given every 2.5 hours, in the next ten days, feed used was in the crumble form given every 3.5 hours, while in the 21^{st} – 28^{th} day, feed used was EP-1 given every five hours. Treatments of candidate probiotic bacteria addition on feed consisting of K7: commercial feed plus isolates K7; K8: commercial feed plus K8 isolates; T36: commercial feed plus isolates T36; T41: commercial feed plus isolates T41; K +: Without the addition of probiotics but infected with Vibrio V6 Rf^R (K +); K-: Without any the addition of probiotics and infected with Vibrio V6 Rf^R (K-).

Challenge test was performed on day 29^{th} using pathogenic *Vibrio* V6 Rf^R, isolates cultured using SWC liquid in shaker speed 180 rpm. Harvesting bacteria were performed according to the time achieving exponential phase (10 hours). The bacterial culture obtained was measured value of density or optical density (OD) to determine bacterial population using a spectrophotometer. Challenge test was conducted using the bacterial pathogen *Vibrio* with a concentration of 10^6 $CFUmL^{-1}$ by the method of soaking for 24 hours. Fish that have been infected then transferred to a new container and maintained for 7 days. Parameters observed in the study included:

Survival Rate Fry: The survival rate fry was calculated using the formula [13].

Growth Rate of Fry: Growth in length and weight of fry was based on the formula of [14]

Bacterial Populations: Measurements of bacterial populations of liver organ was performed after challenge with *Vibrio* pathogens (V6 Rf^R). Observation of pathogenic bacteria *Vibrio* V6 Rf^R population in the liver was performed every 6 hours for 48 hours.

Data Analysis: The data obtained were analyzed using completely randomized design (CRD) at the 95% confidence interval. Further analysis of the results was tested by Duncan test to determine differences between treatments [15].

RESULTS AND DISCUSSION

Bacterial Populations: Populations of *Vibrio* pathogen V6 Rf^Ron the liver organ of tiger grouper fry after challenge test were presented in Figure 1 and 2. All populations of *Vibrio* pathogenV6 Rf^R on tiger grouper fry added probiotics declined started at the 12^{th} hour after challenge test, whereas the positive control population *Vibrio* pathogenV6 Rf^R treatment was increased until the end of the observation.

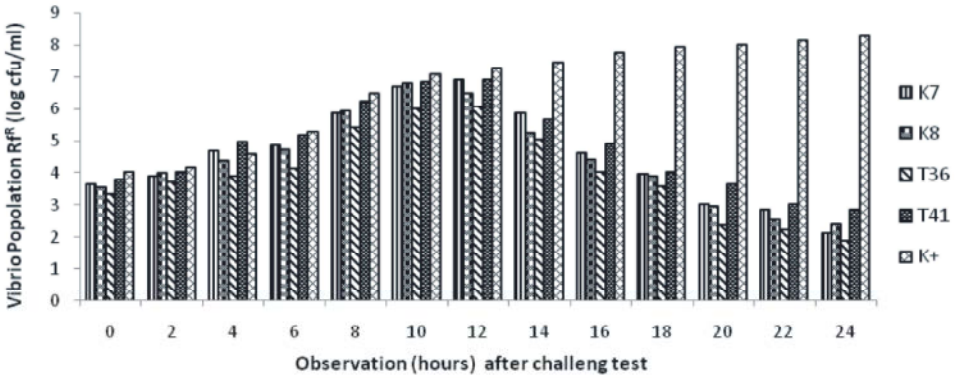

Fig. 1: Population *Vibrio* pathogen V6 Rf^R on rearing of the tiger grouper fry

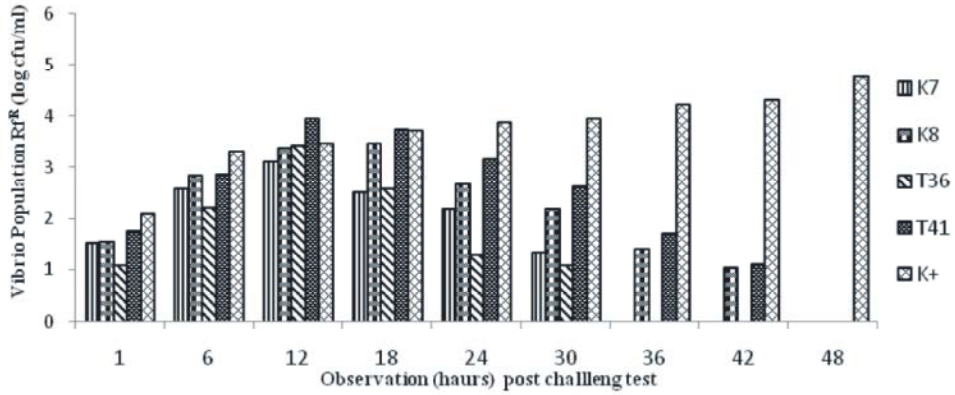

Fig. 2: Population bacterium *Vibrio* V6 Rf^R in the tiger grouper fry liver

Overall the population of *Vibrio* pathogen V6 Rf^R with the addition of probiotics in the treatment of liver tiger grouper fry decreased from the 18th hour post-challenge test. This showed that after 18 hours post-challenge test, the application of probiotic bacteria can reduce populations of *Vibrio* pathogen V6 Rf^R in the tiger grouper liver.

Populations of pathogen *Vibrio* V6 Rf^R in the liver of tiger grouper frry of treatment with the addition probiotic bacteria were lower than the control (without the addition of probiotics). Moreover, population pathogens Vibrio V6 RfR were not detected at the 36th hour in K7 and T36 treatments as show on Figure 2. No detection bacteria pathogen *Vibrio* V6 Rf^R at the 36th hour could be because of a decline in populations of pathogen *Vibrio* V6 Rf^R in the grouper liver fry (due to inhibition of the growth pathogen *Vibrio* V6 Rf^R by probiotic bacteria) so that the opportunity for colonizing of pathogen *Vibrio* V6 Rf^R in tiger grouper fry was smaller in treatment with the probiotic application compared to the treatment without the addition of probiotics.

Another possibility was the competition on attaching to host or nutrient sources [3]. This suggests that the increase in the survival with the addition of probiotics in the treatment. The low density of pathogen *Vibrio* V6 Rf^R prevent number of V6 needed to express virulence factors that can then kill the tiger grouper fry. On the positive control treatment, bacterial pathogen *Vibrio* V6 Rf^R still was detectable up to 48 hours (end of observation). This is possible due to the absence of competition between the bacterial pathogen *Vibrio* V6 Rf^R and probiotic bacteria on rearing and on the liver of tiger grouper fry.

It has been reported five candidate probiotics (AP1-AP5) isolated from fish *Amphiprion percula* (Lacepede) successfully attach and colonize to the mucus of fish, so it can reduce the population of pathogen bacteria *Aeromonas hydrophlla* and *V. alginolyticus* [16] The growth of *V.aguillarum* can be inhibited the growth of *P. fluorecens* AH2 on rearing with limited iron concentration, producing siderofor and antibacterial so that inhibit the growth of *V.anguillarum* [17].

One of the possible mechanisms to prevent colonization of pathogenic bacteria by adding a host of other bacteria that can compete with pathogenic bacteria in dominating attaching point [3, 18, 10]. It has been states not only providing probiotic bacteria to suppress the growth of pathogenic bacteria, but also increase the rate of growth of aquatic animals [19].

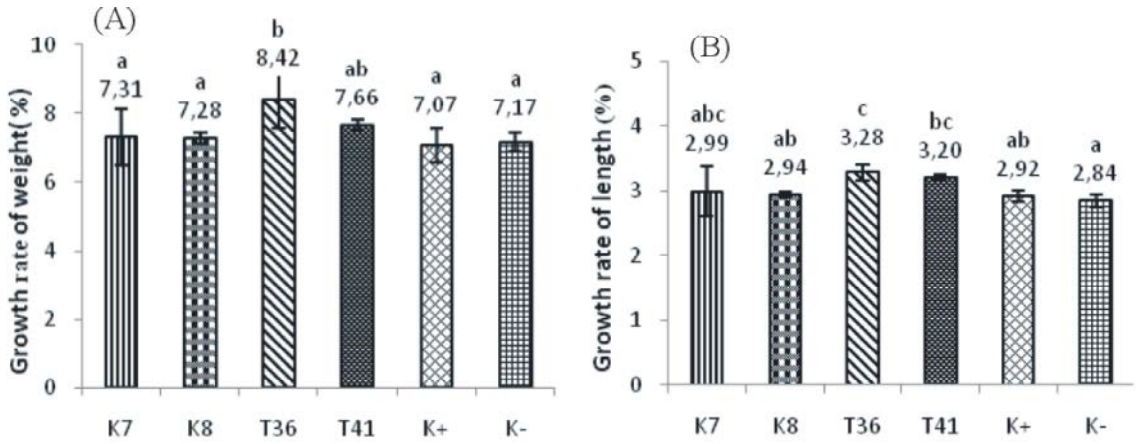

Fig. 3: The growth rate of weight (A) and length (B) of tiger grouper fry after administration of probiotics for 28 days

Growth Rate: Growth rate of tiger grouper fry after administration of probiotics for 28 days was presented in Figure 3. The growth rate weight of tiger grouper fry by administering probiotic bacteria T36 providing the highest weight growth rate compared with other treatments was 8.42% and significantly different (P <0.05) with another treatment and control except in the treatment with the addition of T41 probiotics were presented in Figure 3 A. The growth rate of lenght tiger grouper fry also obtained the highest at addition of probiotic treatment with T36 was 3.28%. Addition of probiotic treatment showed a significantly (P <0.05) with K8 treatment, positive and negative controls were presented in Figure 3B.

The increase is also suspected due to the contribution by the digestive enzymes probiotic bacteria that can improve digestive activity. [20] stated that the digestive enzymes produced by bacteria will improve the digestive process of aquaculture species. This is similar to the opinion of [21, 22] which states that the presence of probiotics in the digestive tract may increase the activity of enzymes that are able to maximize the digestive tract, in addition to an increase in growth can be caused also by an increase in feed nutrients (especially protein). Bacteria is one of the sources of microbial proteins that provide the bacteria in feed to increase protein feed.

Proteolytic bacteria are bacteria that are able to produce proteases that remodel exoenzim proteins into amino acids. Proteolytic bacteria will utilize amino acids as a source of carbon and energy. Lipolytic bacteria are bacteria that are capable of producing exoenzim triglyceride lipase to digest and produce long-chain fatty acids and glycerol used as carbon sources and glycerol, whereas amylolytic bacteria are bacteria that produce amylase to degrade starch into maltose and glucose as a source of carbon and energy [23, 24].

It has been reported that cellulase endogenous enzyme secreted in the digestive tract anterior carp fish, while the remaining cellulose absorption occurs in the posterior part of the digestive tract and indicates the presence of microbes producing cellulase in the segment [25] According to [26], pancreatic lipase is the main enzyme involved in the digestion of triglycerides in all vertebrates.

Probiotics also serves as a source of nutrients and digestive enzymes. Several studies have shown that microorganisms have a beneficial effect on the digestive process in aquatic animals that contribute nutrients, as a source of food supplements, a source of vitamins and essential amino acids [6], affecting digestive activity [7-9]. All information about the enzyme lipase in the intestine of fish are the endogenous enzyme.

Survival Rate: The survival rate of tiger grouper fry after administration of probiotics for 28 days as show on Figure 4 A, the highest obtained was 88.33% in the treatment with the addition of probiotic bacteria T36 and followed on adding probiotic bacteria T41, K8, K7, K + and K- that were 86.67%, 71.67%, 70.00%, 65.00% and 61.67%, respectively. The addition of probiotic bacteria T36 showed survival rates significantly different (p <0.05) with that of K7 and negative control, while did not have a significant effect (p> 0.0,5) with that of the others.

After challenge test, the highest survival rate of tiger grouper fry also obtained was 78.33% on treatment with the addition of probiotic bacteria T36, followed on treatment of T41, K-, K8 and K + as show on Figure 4 B. The observation post challenge test showed survival rates in the addition of probiotic T36 and T41 differed significantly (p <0.05) with the positive control.

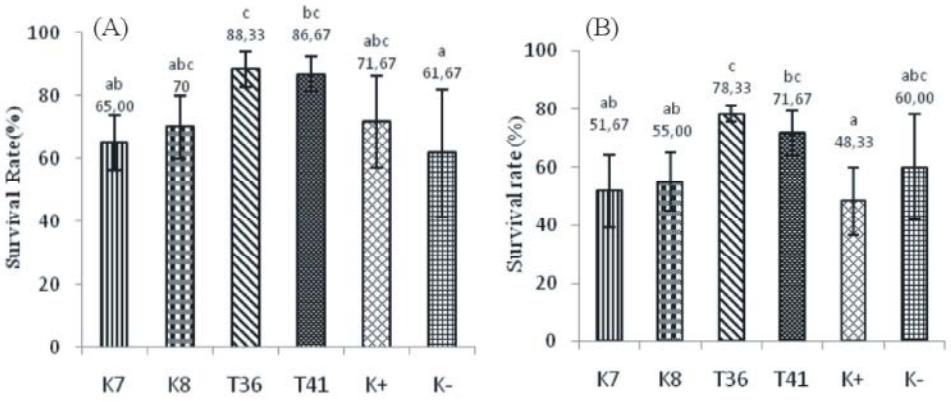

Fig. 4: The survival rate of tiger grouper seed after probiotic treatment for 28 days (A) and 14 days after challenge test (B)

The addition of probiotics in the diet can increase the absorption of nutrients in the body of the fish, the same thing is expressed [3] that the presence of probiotic in the digestive tract can improve microbial balance in the digestive tract thus increasing the absorption of feed and reducing the number of pathogens in the gastrointestinal tract. [4] claimed the presence of probiotics in the digestive tract can increase the synthesis of vitamins and cofactors that are able to maximize the activity of digestive enzymes in the digestive tract.

The results of this study indicated that the addition of probiotic bacteria T36 treatment was more effectively to improve the ability of fish to face the attack of bacteria *Vibrio* V6 Rf^R than the positive control. This is in line with the statement of [10] that probiotics is an agent capable of preventing damage to the host caused by pathogens in general through the competition, but largely by producing substances that can inhibit the growth of harmful microorganisms

CONCLUSION

The addition of probiotic bacteria K7, K8, T36 and T41 can improve survival tiger grouper fry, suppress populations of pathogenic bacteria *Vibrio* V6 and enhance the growth of tiger grouper fry. The addition of probiotic bacteria T36 provedes the best results compared to other isolates. Addition of probiotics can suppress population of *Vibrio* pathogen and incresed the surviral rate of tiger grauoper fry. The use of probiotics can increase the production of tiger grouper.

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