Efficacy of attenuated bacteria vaccine against streptococcal infection in larvae (Oreochromis niloticus)

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ABSTRACT. One of the common diseases found in tilapia is a streptococcal disease caused by the Streptococcus agalactiae bacteria. Treatment of this disease is generally done by using antibiotics. The use of antibiotics in controlling disease in fish can create resistant bacterial strains with certain doses. The purpose of this study was to analyze the efficiency of vaccination on seeds through immersion using attenuated active bacteria to increase immunity at the beginning of the spread of streptococcosis disease. The vaccine used was obtained from attenuated Streptococcus agalactiae bacteria through repeated passage ten times. Vaccination was carried out through oral vaccine. Challenge tests were carried out on days 7, 14, and 21 days after vaccination on tilapia larvae by immersion. The result indicated that vaccination using attenuated bacteria could increase lysozyme activity at each observation time compared to control. The mortality of post-challenged larvae on days 7, 14 and 21 post-vaccination was lower than that of unvaccinated seed. The percentage of RPS values up to the 14th day of observation has increased and tends to decrease on the 21st day.

1. Introduction

Tilapia is one of the aquaculture commodities that is starting to become more popular. This is due to the increasing demand from consumers for this fish. However, the main obstacle for these fish cultivators is the high mortality rate due to disease at the beginning of stocking. One of the common diseases found in this fish is a streptococcal disease caused by the Streptococcus agalactiae bacteria. Treatment of this disease is generally done by using antibiotics. However, the use of antibiotics in the treatment of fish is currently being controlled by the government. The use of antibiotics controlling disease in fish can create resistant bacterial strains. The use of vaccines is one of the alternative control methods in fish defense against diseases caused by disease-causing pathogenic organisms (Akbary et al., 2015).

Several studies have reported the spread of S. agalactiae bacteria in fish in Indonesia, especially in West Java, East Java, Sulawesi, and Papua (Lusiaustiti et al., 2009; Lusiaustiti et al., 2014; Anshary et al., 2014). Furthermore, in the laboratory, the bacterium S. agalactiae is acute in tilapia and indicates that this bacteria can cause more serious streptococcal diseases in tilapia culture (Taufkhi et al., 2012; Liet et al., 2015). Vaccination in fish is believed to reduce mortality due to infection with potential pathogens, reduce the use of antibiotics, and minimize the emergence of resistance of microorganisms to antibiotics (Taufkhi et al., 2012). This is very important considering that government programs to increase aquaculture production need to be supported by the development of vaccination to control disease in fish during the cultivation process. Previously, researchers had succeeded in proving that giving vaccines to tilapia broodstock provided better resistance in seeds to Streptococcosis disease (Sukendra et al., 2018).

In addition to the killed vaccine, live attenuated vaccines are highly considered for scientists considering the several advantages this method provides, including ease to culture, low production costs, and no genetic background (Mohd-Aris et al., 2015). The efficacy test of the attenuated Streptococcus agalactiae HN011 vaccine on tilapia on different routes showed that oral vaccination provided a higher Relative Percent Survival (RPS) value compared to immersion vaccination (Li et al., 2015). The advantage of oral vaccination using attenuated bacteria compared to using...
inactivated bacteria as antigens are that live bacteria can avoid digestive enzymes to reach organs in the fish body and stimulate fish immunity in a sustainable manner (Chen et al., 2015). Furthermore, oral vaccines using attenuated bacteria can stimulate the body to produce mucus as a simultaneous immune response (Makesh et al., 2015). Economically, the use of vaccines in preventing disease transmission is believed to be able to provide more benefits to farmers. The success of fish farming is inseparable from the quality of the seeds used. Fish seeds that have been vaccinated have been shown to have better body resistance than those who have not received the vaccine (Haniifet al., 2005; Akbary et al., 2015). However, vaccination by injection into fish larvae is difficult, so it requires the development of appropriate methods. Previously, the results of some studies had succeeded in increasing the body's resistance to tilapia fish through maternal immunity against infection with Streptococcus agalactiae bacteria. However, antibodies decreased with the increasing age of fish (Nisaa et al., 2016). Therefore, it is deemed necessary to conduct research related to the vaccination of seeds through immersion using attenuated active bacteria to increase immunity at the beginning of the spread of streptococcal disease.

2. Material and Methods

2.1. Material

The material used in this study was tilapia originating from the Brackish Water Cultivation Fisheries Centre (BRBPAB) Takalar, South Sulawesi. The tools used in this study were Petri dishes, incubator, multi-well plate, 1.5 liter volume container, aerator set, 2-meter diameter round tank in the pond, and pellet feed with a protein content of 25% for seeds and 34% for prospective broodstock fish.

2.2. Fish preparation

Tilapia broodstock was placed in a pond with a ratio of 1:3 between males and females. The weight of fish was between 200-300 grams per fish. Feed is given two times a day at satiation. The eggs produced from the mother tilapia were collected and then incubated in an aquarium medium measuring 40x25x20 cm3 with a temperature of 28-29°C; pH 6.78-7.98; and OD 4.5-6.2 ppm. The eggs are kept until they hatch and develop into tilapia larvae ready for testing.

2.3. Attenuated Bacteria Vaccine Preparation

Streptococcus agalactiae bacteria were cultured in a 5% sheep blood agar plate and cultured at 28°C for 24 hours (Liet al., 2015). Subsequently, single colonies were inoculated into 10 mL of TSB (Tryptone Soy Broth) medium and cultured (shaking) at 25°C for 12 hours. 1 mL of bacteria was inoculated into 10 mL of new TSB medium and cultured again (shaking) for 12 hours. Repeated passages were carried out ten times (Li et al., 2015).

2.4. Vaccination of larvae

Seeds were vaccinated orally using S. Agalactiae-N bacteria (product of S. Agalactiae attenuated bacteria). Bacterial isolates with a 1 x 10^7 CFU/ml concentration in PBS were distributed homogeneously on the feed until absorbed, then dried at room temperature for 15 minutes, and given once to fish (Liet al., 2015). The control treatment in this study was larvae without vaccination. The vaccinated seeds were challenged with homologous bacteria and observed on the 7th, 14th, and 21st days after vaccination. Each treatment in this study used as many as 20 fish seeds.

2.5. Homogenate Preparation of Larvae

Larvae in each treatment was collected. The collected larvae were rinsed three times using sterile PBS pH 7.2, then homogenized in a solution of Phosphate-Buffer Saline plus 0.05% tween-20 (PBS-T) four times the volume and centrifuged at 5000 g for 10 minutes at temperature 4°C. The supernatant was collected, then centrifuged twice at 5000 g for 5 minutes and then stored at -20°C until the analysis was carried out (Akbary et al., 2015).

2.6. Immunological Assay

2.6.1. Lysozyme Activity

Lysozyme activity of larvae tilapia was measured by a method based on ability of lysozyme to lyse the bacterium Micrococcus lysodeikticus (Haniifet al., 2004). Homogenate larvae (100 μL) were put into a microtiter plate, and suspension of Micrococcus lysodeikticus bacteria (100 μL) was added in phosphate buffer with a concentration of 0.4 mg ml^-1. Next, the microtiter plate was incubated at 22°C, and the optical density (OD) was read at 450 nm. OD readings were taken at 30 seconds and 30 minutes.

2.6.2. Mortality and Relative Percent Survival (RPS)

Seed mortality rates were calculated on day 14 post-challenge with homologous bacteria. The seed mortality rate is calculated using the following formula:

\[
\text{Mortality} (%) = \frac{\text{number of dead fish}}{\text{number of population}} \times 100 \hspace{1cm} (1)
\]

The RPS of tilapia seeds after the challenge test with homologous bacteria was calculated to determine the effectiveness of tilapia seed vaccination. The RPS value is obtained using the following equation (Amend, 1981): .

\[
\text{RPS} (\%) = (1 - \frac{\text{cum. mortality vaccinated group}}{\text{cum. mortality control group}}) \times 100 \hspace{1cm} (2)
\]

2.7. Data analysis

The data obtained were tabulated with the MS Office Excel 2013 program and analyzed using ANOVA through the Minitab version 16 program with a 95% confidence level. If significant (P < 0.05), it would be further tested with Tukey's test.

3. Results and Discussion

3.1. Lysozyme Activity

Based on the measurement results of post-vaccination lysozyme activity, it is known that the trend of lysozyme activity in vaccinated tilapia larvae was higher than control at each observation time (Figure 1). This indicates that the vaccine can increase lysozyme activity in tilapia larvae given a live attenuated vaccine through a repeated passage. The enzyme lysozyme is one of the essential bacterial enzymes in the innate immune of fish. This enzyme functions in warding off disease attacks in some fish (Lusiaet al., 2009). According to Akbary et al. (2015), lysozyme is a bactericidal enzyme involved in the hydrolysis of the (1,4) glycoside bonds of the peptidoglycan chain from the bacterial cell wall so that the bacterial cell undergoes lysis.

![Figure 1. Lysozyme activity of tilapia seeds after vaccination. Each data point represents the mean (±S.E) of triplicates. Groups significantly different (P<0.05).](https://www.sangia.org/)
3.2. Mortality Rate and Relative Percent Survival of tilapia fry after the challenge test

Based on the results in Figure 2, it is known that the mortality caused by streptococci disease after challenge with homologous bacteria in vaccinated seeds decreased on the 14th day of observation and increased up to 18.3% on the 21st day of observation. Obtained is still much lower than the mortality found in control (non-vaccinated larvae). Based on the statistical analysis results, it was found that the mortality caused by streptococcosis was significantly different at each time of observation in the treatment of vaccinated and control fish fry (P<0.05).

![Figure 2. Mortality of tilapia seeds post challenge test. Each data points represents the mean (±S.E) of triplicates. Groups significantly different (P<0.05).](image)

Vaccine efficacy was defined as the proportional relationship between vaccinated and unvaccinated fish mortality groups after being challenged with pathogenic bacteria. Vaccine efficacy can be proven by measuring the RPS value (Gudding, 2014). The lowest value of RPS was found on the 7th day of observation, then increased on the 14th day, indicating that this vaccine was able to provide fitness to tilapia fry and prevent death from death streptococcosis. Previous researchers have also shown that administering a vaccine using an attenuated strain of Streptococcus agalactiae HNO11 bacteria in tilapia on different routes showed that oral vaccination gave a higher Relative Percent Survival (RPS) value compared to vaccination through immersion (Lusiastrutti et al., 2014).

![Figure 3. Relative percent survival of tilapia seeds post challenge test. Each data points represents the mean (±S.E) of triplicates.](image)

The advantage of vaccination using attenuated bacteria compared to using inactivated bacteria as antigens are that live bacteria can avoid digestive enzymes to reach organs in the fish body and stimulate fish immunity on an ongoing basis (Anshary et al., 2014). Furthermore, oral vaccines using attenuated bacteria can stimulate the body to produce mucus as a simultaneous immune response (Taukhid et al., 2012). Economically, the use of vaccines in preventing disease transmission is believed to provide more benefits to farmers.

In Figure 3, the percentage of RPS decreased on the 21st day. This indicates a reduction in the protection ability of fish fry from streptococcal disease. This can be the basis for further research. It is necessary to conduct a booster trial using an attenuated vaccine to increase the ability of the seeds to protect themselves from streptococcosis infection at the beginning of the stocking period in the cultivation process.

4. Conclusion

The vaccine using attenuated Streptococcus agalactiae bacteria protected tilapia fry until the end of the study. However, the decrease in the RPS value at the end of the study indicated that the seeds needed a booster vaccine to increase their ability to prevent the spread of streptococcosis in tilapia fry.

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Supplementary files

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study, and/or contains supplementary material, which is available to authorized users.

Competing interest

All author(s) declare no competing interest.

Reference


