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Efficacy of Purple Non-sulfur Bacterium *Afifella marina* Strain ME to Control Dissolved Inorganic Nutrients in Aquaculture System

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ABSTRACT

Experiment was conducted to determine the possibility of using locally isolated purple non-sulfur bacterium *Afifella marina* strain ME to improve the dissolved inorganic nutrients (DIN) in Tilapia *Oreochromis niloticus* culture tank. The experiment was conducted for seven days without changing water. Ammonia (mg/L), nitrite (mg/L), nitrate (mg/L) and phosphate-phosphorus (mg/L) in the Tilapia culture tank were monitored. Sixteen tails of *Oreochromis niloticus* juveniles with mean weight of 0.7±0.05g were stocked in ten liter aquarium. Juveniles were fed with commercial feed twice daily by *ad-libitum* feeding method. Purple non-sulfur bacterium *Afifella marina* strain ME, and established probiotic commercial *Bacillus* with four inclusion levels, 0.005(g/L), 0.01(g/L), 0.02(g/L), and 0.03(g/L) were added everyday into culture tank. At the end of experiment no significant difference ($P > 0.05$) were observed among all the inclusion levels with the concentration of ammonia, nitrite, nitrate and phosphate. The lowest concentration of ammonia, nitrite, nitrate and phosphate were observed in both *Afifella marina* strain ME, and commercial *Bacillus* with the inclusion level of 0.03g/L. Obtained results were comparable with commercially established probiotics *Bacillus* sp. Locally isolated purple non-sulfur bacterium *Afifella marina* strain ME could be one of the potential candidate in controlling dissolved inorganic nutrients in aquaculture system.

Introduction

One of the constraints in aquaculture is to maintain the water quality problem suitable for farming. Aquaculture with high stocking density must have to use high-protein formulated feed to optimize the growth and survival in intensive culture system. Fish farming wastes contribute to dissolved nitrogen and phosphorus but not to silica (Mente *et al*., 2006). Excess deposition of nutrient from feed and fish waste will deteriorate water quality in aquaculture system. It directly influences to the dissolved oxygen concentration, pH of water, phytoplankton abundance and also development of opportunistic bacteria in water environment. The dissolved inorganic nutrients such as ammonia, nitrite, nitrate and phosphate phosphorus etc. will also be increased. The components such as ammonia and nitrite with excess are harmful components to the aquaculture species. Therefore there is a need to maintain the water quality suitable for aquaculture species in terms of reducing the quantity of ammonia, nitrite, phosphorus and organic matters in the culture system since the water quality is the limiting factor for better growth and survival. Inclusion of probiotics is one of the options to control the inorganic and organic harmful matter generated in aquaculture system. Bacteria species such as *Bacillus* sp., *Pseudomonas* sp., *Rhodoseudomonas* sp., *Nitrosomas* sp. and *Nitrobacter* sp., etc. have been applied for improvement of water quality in aquaculture (Verschuere *et al.*, 2000). Thus, searching and build-up of an excellent probiotics microflora distribution in aquaculture system are essential. Besides levels of inclusion and application techniques are important in order to identify the effectiveness of probiotics in aquaculture system. This study was conducted to determine the effectiveness of using locally isolated purple non-sulfur bacterium *Afifella marina* strain ME to control the dissolved inorganic nutrients (DIN) in Tilapia *Oreochromis niloticus* culture tank.

Methodology

Purple non-sulfur bacterium *Afifella marina* strain ME, and established probiotic commercial *Bacillus* with four inclusion levels were used as treatment, while aquarium without bacteria used as control. *Afifella marina* was obtained from the culture collection of Borneo Marine Research Institute (BMRI), University Malaysia Sabah, Malaysia. *Afifella marina* strain ME was cultured in two liter Schott bottle together after adding 10% of inoculum in 112 media and incubated anaerobically under the light intensity of 2500 lux at a temperature of 30°C ± 2°C for 36 hours. After 36 hours biomass of the *Afifella marina* was harvested by centrifugation process and stored until start of experiment. Commercial *Bacillus* powder (AQUAPRO-EZ Aquaintech Inc) was obtained from local market. This powdered material contains at least 4 billion CFU per gram. Desired amount of commercial *Bacillus* powder was measured and activated through strong aeration for 20 minutes before introduced into experimental tanks. *Oreochromis niloticus* mean weight of 0.07±0.5g were obtained from the UMS hatchery and acclimatized for one week before the start of the experiment. Fishes were randomly allocated into 27 aquariums (nine treatments with three replicates and working volume 7L) with 16 tails per tank. *Oreochromis niloticus* juveniles were fed with commercial diet two times daily by *ad-libitum* feeding method. Four different amount of commercial *Bacillus* and *Afifella marina* strain ME, which were at weight of 0.005g, 0.01g, 0.02g, and 0.03g were activated everyday through strong aeration of water before inclusion into the experimental tank. Dissolved inorganic nutrients in aquarium water such as total ammonia (mg/L), nitrate (mg/L), nitrites (mg/L) and phosphate (mg/L) was determined everyday through spectrophotometric method described by Parsons *et. al*. (1984). The duration for this experiment was seven days without changing of aquarium water.

Result and discussion

The lowest concentration of 2.45mg/L and 2.58mg/L of ammonia was determined at inclusion level of 0.3g/L of *Bacillus* sp. and *Afifella marina* respectively at the end of experiment. All the treatments were not significantly different from each other ($P > 0.05$) (Figure 1).

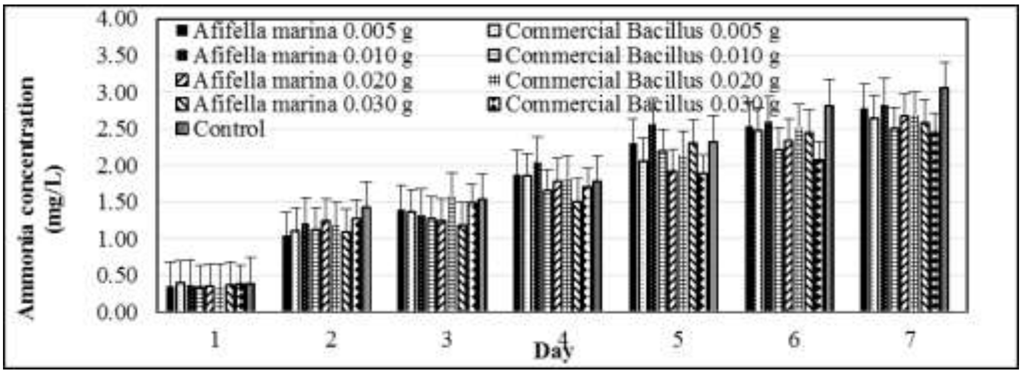

Figure 1. Changes of ammonia concentration (mg/L) in the *Oreochromis niloticus* culture system during experimental period

The concentration of nitrite in the experiment tank always was observed lower with the application of *Bacillus* sp (Figure 2). However, the lowest concentration of 0.019mg/L and 0.021mg/L nitrite were recorded at inclusion level of 0.3g/L with *Bacillus* sp and *Afifella marina* strain respectively. The lowest concentration achieved with these 0.3g/L inclusion level has observed to significantly different ($p<0.05$) then the other inclusion levels at day 7.

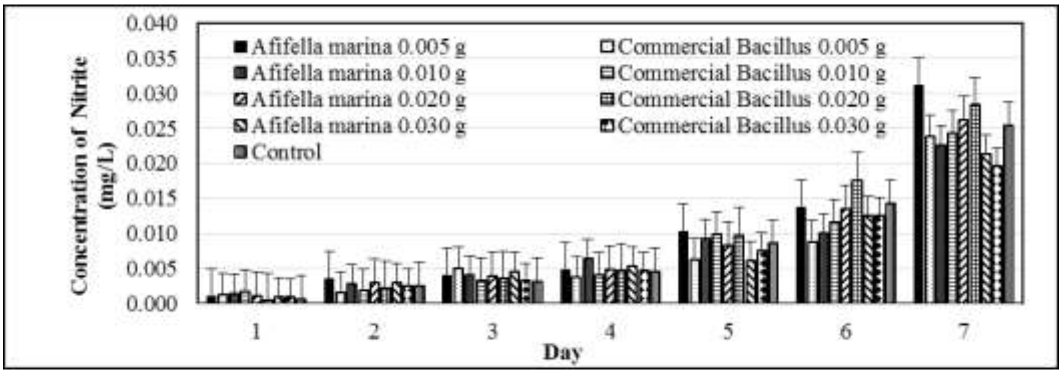

Figure 2. Changes of Nitrite concentration (mg/L) in the *Oreochromis niloticus* culture system during seven days experimental period

The highest concentration of 0.019mg/L and 0.016mg/L of nitrate were observed at 0.005g/L inclusion level of *Bacillus* sp and *Afifella marina* strain, respectively. On the other, hand the lowest concentration of 0.008mg/L and 0.009mg/L of nitrate were acheived at 0.03g/L inclusion level of *Bacillus* sp and *Afifella marina* strain, respectively (Figure 3). At the end of experiment treatment, the 0.03g/L of commercial *Bacillus* did show significant different ($P < 0.05$) with all treatments expect for the treatment 0.03g/L inclusion level of *Afifella marina*.

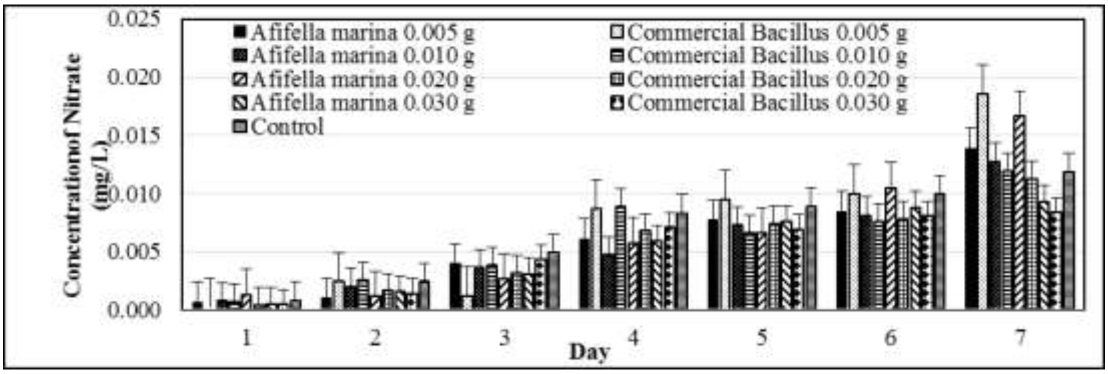

Figure 3. Changes of concentration of nitrate (mg/L) in the *Oreochromis niloticus* culture system during seven days experimental period.

The highest concentration of 0.772 mg/L phosphorus was obtained in control (without bacteria) treatment. The increasing trends of phosphorus were observed from day four to seven, however the lowest concentration always observed with *Bacillus* sp at 0.03 g/L inclusion level (Figure 4). At day seven, treatment with 0.03g/L of commercial *Bacillus* showed significant different ($P < 0.05$) with all other treatments.

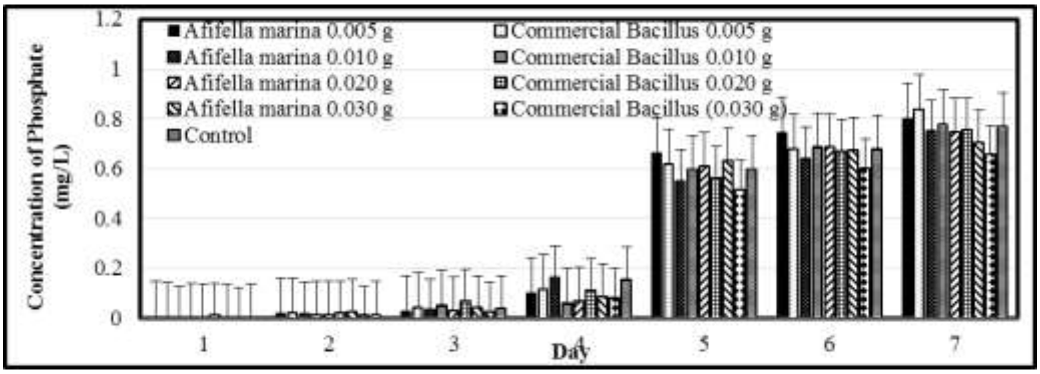

Figure 4. Changes of phosphate phosphorus (mg/L) in the *Oreochromis niloticus* culture system during seven days experimental period

Probiotics can help to improve the water quality for the aquaculture use. Water quality can be improved through improving the water chemistry content, such as eliminating or reducing the ammonia, nitrite, nitrate, organic matter content (Balcázar *et al.*, 2006). The concentration of dissolved inorganic nutrient parameters, like ammonia, nitrite, and nitrate and phosphate level showed an increasing trend day by day in this experiment. Numerically, lower inclusion level always showed higher concentration of studied parameters while inclusion with 0.03g/L of commercial *Bacillus* and *Afifella marina* helped to reduce the ammonia concentration to a lowest concentration levels. Concentration of ammonia-nitrogen for juvenile *Oreochromis* sp. must be below 3.0mg/L (Zhou *et al.*, 2010). However, Tilapia started to die at ammonia concentration around 2 mg/liter. Ammonia production depends on quality of feed, feeding rate, fish size and water temperature. Fish weight decreased when concentrations of unionize ammonia increased. It was attributed to a decrease in daily food intake, daily feed consumption and decrease in food conversion efficiency. Tilapia is the most tolerant species to ammonia. Effects of adding *Rhodobacter sphaeroides*, *Rhodobacte*r *capsulatus* and *Rhodopseudomonas palustris* in prawn feed during grow-out culture fed to shrimp had shown increased weight compared to control commercial diet (Qiao *et al*., 1994). They also observed improved water quality when fed with phototrophic bacteria, as ammonical nitrogen was observed to be significantly lower with control diet. Pond ecosystem could be improved by adding phototrophic bacterial biomass. Phototrophic bacteria could convert sulfite to sulfate and have high sulfur tolerance capacity. Phototrophic bacteria also have a strong nitrogen fixing ability (Qiao *et al*., 1994).

Wickins (1981) suggested that nitrite concentration in fish culture tank should not exceed 0.1 mg. In this trial also, the nitrite concentration from all treatments did not exceeded 0.1 mg/l, at which the lowest level of nitrite concentration of achieved with both *Afifella marina* and *Bacillus* sp at 0.03g/L inclusion level. Nitrite (NO_2) is an intermediate step in nitrification of ammonias (NH_4^+ and NH_3) to nitrate (NO_3). In aquaculture waters nitrite forms primarily as a byproduct of microbial metabolism, and to a much lesser extent, by chemical oxidation/reduction reactions (Wong, 1989). Nitrite enters a fish culture system after feed is digested by fish and the excess nitrogen is converted into ammonia, which is then excreted as waste into the water. Total ammonia nitrogen (TAN; NH_3 and NH_4^+) is then converted to nitrite (NO_2) which, under normal conditions, is quickly converted to non-toxic nitrate (NO_3) by naturally occurring bacteria. The nitrifying bacterial colonies appeared to be well established in culture system, so nitrite concentration declined to less than 1.0 mg/l. The maximum NO_2-N concentration of 2.7 mg/l is comparable with the 2.7–3.2 mg/l for *O. niloticus* fingerlings using rotating biological filter (Ridha & Cruz, 2001).

Nitrate concentration in this trial showed a huge increasing trend in the treatments, but the lowest values obtained with both *Afifella marina* and *Bacillus* sp at 0.03g/L inclusion level. Nitrification process from commercial *Bacillus* and *Afifella marina* bacteria in the experimental tanks might work efficiently at a short period of time. However, there will no negative effects to fish have been reported at nitrate level below 100 mg/L (Wickins, 1981). Nitrite is apparently not directly assimilated by aquatic plants as a source of nitrogen for protein synthesis. Nitrate toxicity can occur if levels in water reuse systems exceed the 300 to 400 mg/L nitrate-nitrogen range (Wickins, 1981). Nitrifying cultures could also be added to the ponds or the tanks when an incidental increase of ammonia or nitrite levels was observed (Balcázar *et al.*, 2006).

The highest concentration of 0.84 mg/L phosphate was observed in treatment of 0.005 mg/L of commercial *Bacillus* inclusion and the lowest with same bacterium with 0.03g/L inclusion level. Phosphate generally did not give negative impact to the physiological of the aquaculture organism. Gram-positive bacteria in the water environment especially in the water bodies with phytoplankton inclusion can increase the organic carbon with supplying those organic carbon from conversion of organic matter in order to promote a stable phytoplankton blooms (Verschuere *et al*., 2000). *Afifella marina* is the gram-negative bacteria and can able to survive under anaerobic and aerobic condition. Although no detail study was conducted to observe the role of *Afifella marina* in the utilization for phosphate, but other purple non-sulfur bacterium *Rhodospeudomonas palustris* is believed can used organophosphorus and can improve the water quality for aquaculture industries (Berne *et al*., 2007). They also stated that *Rhodospeudomonas palustris* have the ability to degrade the tributyl phosphate (TBP), which it is toxic organophosphorus compound that causes severe environmental pollution and human health problems due to release of tributyl phosphate.

In several studies, water quality has been recorded during the addition of the probiotics, especially *Bacillus* spp. It is reasoned that by maintaining higher levels of these gram positive bacteria in the production pond, farmers can minimize the buildup of dissolved and particulate organic carbon during the culture cycle (Verschuere, 2000). However, several studies utilizing one or more bacterial species such as *Bacillus*, *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, and *Rhodopseudomonas* spp. in the culture of shrimps or channel catfish could not confirm this hypothesis (Balcázar *et al*., 2006). A lot of bacterial cultures containing nitrifying bacteria to control the ammonia level in culture water are available commercially and are aimed especially at aquarium hobbyists. Nitrifies are responsible for the oxidation of ammonia to nitrite and subsequently to nitrate. The efficacy of purple non-sulfur bacteria as potential probiotic is still in initial stage and requires further investigation.

Conclusion

Commercial *Bacillus* have better effectiveness in improving water quality, but did not achieve the desired level. Similarly *Afifella marina* strain ME unable to reduce the dissolved nutrients level, but comparable with the commercial *Bacillus* sp. So, further studies are necessary to optimize the amount of commercial *Bacillus* sp. and *Afifella marina* strain ME in order to have better control on dissolved inorganic nutrients in aquaculture system.

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