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The effects of a marine photosynthetic bacteria *Rhodovulum sulfidophilum* on the growth and survival rate of *Marsupenaeus japonicus* (kuruma shrimp)

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ABSTRACT

The sustainability of the shrimp aquaculture depends largely on disease control and the health status of shrimp. Probiotics, which make shrimps healthier and more resistant to pathogens, are promising countermeasure for shrimp diseases. In this study, the effects of the marine purple non-sulfur photosynthetic bacterium (PNSB) *Rhodovulum sulfidophilum* on *Marsupenaeus japonicus* (kuruma shrimp) growth and survival were examined in 177 m^2 aquaria (140 tons of water) for 70 days. The shrimp received feed containing 0.01 % fresh weight (10^6 colony forming unit/g) of *R. sulfidophilum* cells. The survival rate significantly improved ($P < 0.001$) (*R. sulfidophilum*-fed = 81.9 %; control = 71.5 %), the feed conversion rate improved (*R. sulfidophilum*-fed = 1.83; control = 2.11), and there was no difference in the shrimp average body weight. The approximate bacterial cell cost was \$0.003 to \$0.005 per 1 kg feed, indicating that the *R. sulfidophilum* approach is economically feasible and a promising candidate for probiotic bacteria in shrimp aquaculture.

Keywords: photosynthetic bacteria, *Rhodovulum sulfidophilum, Marsupenaeus japonicus*, shrimp, probiotics

1. INTRODUCTION

Shrimp aquaculture is economically important in many countries, and probiotics has attracted attention as an environmentally friendly and cost-effective way to grow healthy shrimps [1]. Probiotic bacteria used in aquaculture are mainly lactic acid bacteria and *Bacillus*, but purple non-sulfur photosynthetic bacterium (PNSB) is also a promising candidate. Shrimps, such as *Litopenaeus vannamei* and *Penaeus monodon* (black tiger), are raised in large quantities in Southeast Asian countries and China, and the PNSB, *Rhodopseudomonas palustris*, is commonly used as a probiotic and water conditioner [2]. *Marsupenaeus japonicus* (kuruma shrimp), a high price shrimp, are cultured in southern Japan. PNSB use is not common in Japanese aquaculture, but *Rhodobacter sphaeroides* (a PNSB) has been used in some shrimp ponds [3]. *Rhodopseudomonas* and *Rhodobacter* are terrestrial (freshwater) PNSB strains. Therefore, marine PNSB strains might be a better probiotic for shrimp because most are grown in seawater or brackish water.

Rhodovulum sulfidophilum is a marine PNSB, which is relatively easy-to-culture with various biotechnological applications, such as biohydrogen production [4], biomaterial production [5–7], and bioremediation [8] . Several studies used *R. sulfidophilum* in aquaculture [9–11], but the cells were added to the feed at relatively high concentrations (> 1 % fresh cell weight) as a nutrient supplement rather than a probiotic. A high dosage of bacterial cells results in high costs, making these approaches economically impractical. Another application of *R. sulfidophilum* in aquaculture is adding *R. sulfidophilum* cells to water recycling aquaculture system to improve the water quality and microbial communities [12].

In this study, the effects of a low *R. sulfidophilum* cell dose (10^6 colony forming units (cfu)/g feed; 0.01 % fresh cell weight) on *Marsupenaeus japonicus* growth and survival were examined in 177 m^2 aquaria (140 tons of water) for 70 days.

2. MATERIALS & METHODS

The marine PNSB strain, *Rhodovulum sulfidophilum* OKHT16 (16S rRNA GenBank/EMBL/DDBJ accession number LC037397), was isolated from Osaka Bay, Japan, seashore sediment as previously described by Yamauchi et al. [13]. This strain is fast-growing (specific growth rate = 0.53 h^{-1} at 36 °C in light), thermotolerant (up to 48 °C), and can assimilate glycerol [13]. *R. sulfidophilum* OKHT16 was cultured in glutamate malate (GM) medium [14] with 3 % sodium chloride (NaCl) in light and aerobic conditions.

Two round outdoor aquaria (15 m diameter, 177 m^2) located in the Fukuyoshi branch of the Fisheries Cooperative Association of Itoshima, Itoshima, Fukuoka, Japan, were used for the experiments. The aquaria bottoms were covered with sea sand (~20 cm deep), and then filled with sand-filtered seawater; the water depth was 80 cm (~140 tons of water per aquarium). Feed numbers 2, 4, and 5 (juveniles) and P1 (adults) from Hayashikane Sangyo Co., Japan, were used depending on the growth stage. *R. sulfidophilum*-containing feed (10^6 cfu/g; 0.01 % fresh cell weight) was prepared by suspending bacterial cells in a 3 % NaCl solution then mixing it with dry feed by shaking. The dosage (10^6 cfu/g feed) was determined based on previous studies with other kinds of probiotic bacteria [15–17]. The feed amount per day was approximately 5 % of the shrimp body weight. Feed leftovers were checked daily, and the amount was adjusted based on the leftovers. Water was circulated with two paddlewheel aerators, and the temperature, pH, and dissolved oxygen (DO) were recorded daily at 08:00 and 15:00. No water change was performed during the first 30 experimental days, after which approximately 30 % of the water was exchanged with fresh seawater once a week.

The experiment ran for 70 days, and on days 10, 20, 30, and 40, the total body weight of 30 shrimp was measured, and the average body weight was calculated by dividing the total body weight by 30. For days 50, 60, and 70, individual measurements were taken, and the average body weight and standard deviation were calculated. The statistical difference in the number of survivors at the end of experiment between *R. sulfidophilum*-fed and control was examined by Chi-squared test.

3. RESULTS AND DISCUSSION

Nine thousand shrimp were initially released into the control and *R. sulfidophilum*-fed aquaria with an average body weight of 0.03 g; the experiment ran for 70 days (April 28 - July 7, 2017). Figure 1 shows the changes in the average body weights of the control and *R. sulfidophilum*-fed shrimp; there was no difference.

Figure 1: Changes in the average body weights of the control (open circle) and *R. sulfidophilium* fed (closed circle) shrimps. On days 10, 20, 30, and 40, the total body weight of 30 shrimp was measured, and the average body weight was calculated by dividing the total body weight by 30. For days 50, 60, and 70, individual measurements were taken, and the average body weight and standard deviation were calculated.

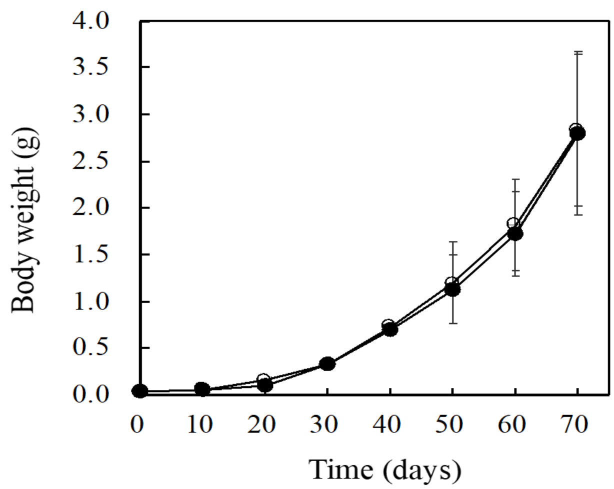

Table 1. Average body weights, number of survivors, survival rates, total harvested body weights, total feed amounts, and feed conversion efficiencies (FCE) for control and *R. sulfidophilum*-fed shrimp.

	Average body weight	Final # of shrimp[1]	Survival rate (%)	Total body weight (kg)	Total feed amount (kg)	FCE[2]
Control	2.83	6434	71.5	18.2	38.5	2.11
R. sulfidophilum-fed	2.80	7372***	82.0	20.6	37.7	1.83

[1] Initial shrimp number = 9000.

[2] FCE (feed conversion efficiency) = (total feed amount) / (harvested total body weight)

*** P < 0.001 (Chi-squared test)

Table 1 shows the shrimp average body weights, number of survivors, survival rates, harvested shrimp total weights, total feed amounts, and feed conversion efficiencies (FCE; total feed amount/harvested total body weight) for the control and *R. sulfidophilum*-fed shrimp after 70 days. There was no difference in average body weight (2.83 g (control); 2.80 g (*R. sulfidophilum*-fed)), but the survival rate significantly improved with *R. sulfidophilum* (71.5 % (6434 of 9000) (control); 81.9 % (7372 of 9000) (*R. sulfidophilum*-fed); (P < 0.001, Chi-squared test). The improved survival rate in *R. sulfidophilum*-fed shrimp also increased the total harvested weight (18.2 kg (control); 20.6 kg (*R. sulfidophilum*-fed)) and improved the FCE value (2.11 (control); 1.83 (*R. sulfidophilum*-fed)).

The results showed that *R. sulfidophilum* cells added to *M. japonicus* feed at 10^6 cfu/g (0.01 % of fresh cell weight) acts as a probiotic and is economically feasible for aquaculture. In Japan, the price of commercial pure PNSB culture (1 x 10^9 cfu/mL) is $3 to $5 for 1 L [18]. Adding *R. sulfidophilum* cells to feed at a concentration of 10^6 cfu/g is equivalent to 1 mL of PNSB culture (1 x 10^9 cfu/mL) per 1 kg of feed and costs $0.003 to $0.005; *M. japonicus* feed is approximately $5 per 1 kg. Therefore, the PNSB cost is 0.06 % to 0.1 % of the feed cost, making *R. sulfidophilum* a cost-effective and feasible probiotic for shrimp aquaculture

4. CONCLUSIONS

Rhodovulum sulfidophilum, a marine purple non-sulfur photosynthetic bacterium (PNSB), added to the feed at 10^6 cfu/g (0.01 % of fresh cell weight) acts as a probiotic, and improves the survival rate, total harvested weight and feed conversion efficiencies (FCE) in *M. japonicus* aquaculture. The cost for PNSB cells is 0.06 % to 0.1 % of the feed cost, thus *R. sulfidophilum* is a cost -effective and feasible probiotic for shrimp aquaculture.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

Author's contributions

Aoi Koga cultured PNSB and analyzed the data. Yusaku Tani and Ken-ichi Ozaki designed and performed the aquaria experiment. Takaaki Maki designed the procedure for the preparation of shrimp feed containing PNSB. Shuhei Hayashi and Shinjiro Yamamoto revised the manuscript critically. Hitoshi Miyasaka designed the study and wrote the manuscript.

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