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Aquaculture 252 (2006) 516–524

Aquaculture

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The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*

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Received 19 April 2005; received in revised form 25 July 2005; accepted 25 July 2005

Abstract

This study examined the effects of a commercial *Bacillus* probiotic on the digestive enzyme activity, survival and growth of *Fenneropenaeus indicus* at various ontogenetic stages in three separate experiments: (1) $Nauplius_{1–2}$ to $Zoea_3$, which were exposed to probiotic added directly to the water; (2) $Mysis_1$ to PL_{14} in tanks, which were exposed to the probiotic either through adding it directly to the water or by feeding shrimp with probiotic-enriched *Artemia*; (3) postlarval shrimp reared in earthen ponds during the farming stages (PL_{30} to PL_{120}), which were exposed to probiotic added to the water. The counts of *Bacillus* bacteria in the digestive tract in all treatments were significantly ($P<0.05$) higher than in controls (no *Bacillus* bacteria were detected in any controls), although total bacterial counts were not significantly different among treatments and controls. Colonization rates of shrimp digestive tracts by *Bacillus* bacteria were very low in all treatments in earthen ponds. In most treatments, the specific activities of amylase, total protease, and lipase were significantly higher ($P<0.05$) in shrimp to which probiotic had been administered, and shrimp that had received probiotic exhibited significant ($P<0.05$) increases in both survival (11–17% higher) and wet weight (8–22% higher) as compared to controls. Shrimp fed probiotic-enriched *Artemia* had significantly ($P<0.05$) higher *Bacillus* counts than did shrimp administered probiotic in the water, but growth and survival were not significantly different between the two modes of administration. Where probiotic was administered during both the hatchery stages ($Nauplius_{1–2}$ through PL_{30}) and the farming stages, the feed conversion ratio, specific growth rate, and final production were slightly, but significantly ($P<0.05$), higher in shrimp receiving the probiotic than in control shrimp which had received no probiotic. Because these improvements in growth parameters in postlarval shrimp were significant only in shrimp

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doi:10.1016/j.aquaculture.2005.07.021

that had received the probiotic both during hatchery stages and during farming stages, it appears to be important for the shrimp to receive the probiotic in all ontogenetic stages in order for these improvements to be realized.

Keywords: *Bacillus*; Probiotic; Indian white shrimp; *Fenneropenaeus indicus*; Growth; Survival; Digestive enzyme activity

1. Introduction

The use of probiotics in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999). A probiotic is generally defined as a live microbial food supplement which improves the balance of the host animal's intestinal flora (Fuller, 1989). However in aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1998). Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thompson et al., 1999; Verschuere et al., 2000).

Because *Bacillus* bacteria secrete many exoenzymes (Moriarty, 1996, 1998), these bacteria have been used widely as putative probiotics. Studies have shown that when these bacteria were administered as probiotics in the shrimp *Penaeus monodon*, growth and survival were improved and immunity was enhanced (Rengpipat et al., 1998a,b, 2000). However, the nutritional effects of probiont bacteria, especially the effects of the bacteria on digestive enzyme activity, have not been evaluated in aquaculture. The present study examines the effect of *Bacillus* probionts on digestive enzyme activity, survival and growth in the shrimp *Fenneropenaeus indicus*.

2. Materials and methods

2.1. Rearing of shrimp

Three separate experiments were conducted to examine the effect of probiotics administered to the Indian white shrimp *F. indicus* H. Milne-Edwards, 1837. All treatments and controls were repeated in triplicate. Larvae and postlarvae were obtained from eyestalk-ablated spawners (average weight 40 g). Shrimp nauplii were reared in aerated tanks with 31–32‰ salinity natural seawater supplemented with a mixture of the microalgae *Chaetoceros* and *Tetraselmis*, which was added daily at a rate of 2×10^6 cells/ml. Typically, 10% of the tank water was exchanged each day. Beginning at mysis stage 1 (M_1), shrimp larvae were fed *Artemia franciscana* nauplii at a rate of 3–4 nauplii for each shrimp larva 4 times per day until mysis stage 3 (M_3) and at a rate of 8–10 nauplii for each shrimp larva 5–6 times per day from M_3 through 14 days after metamorphosis (PL_{14}). Although the microalgae were used as food primarily by early larval stages, we continued supplementing the culture tanks with microalgae throughout the hatchery stages, in order to maintain a green-water culture system. After the end of the hatchery stage (PL_{30}), postlarvae were transferred to 100 m^2 earthen ponds with a mean depth of 1.0 m at a rate of 20 postlarvae/m^2. Ponds were filled with 39.0–42.1‰ seawater (average, 41.3‰); 10% of the pond water was exchanged daily. Shrimp in ponds were fed commercial pellets containing 38–42% crude protein (Havourash Co., Boushehr, Iran) at a rate of 7–11% of shrimp body weight per day for the first month and then at 5–6% of body weight per day until harvested.

2.2. Experimental design

The commercial probiotic used in this study (Protexin Aquatech, Probiotics International Ltd, Somerset TA146QE, United Kingdom) contained spores of 5 species of *Bacillus* (i.e., *B. subtilis*, *B. licheniformis*, *B. polymyxa*, *B. laterosporus* and *B. circulans*). Before administration of the probiotic, spores were rehydrated to vegetative bacteria according to manufacturer's instructions (see Ziaei-Nejad, 2004, for details). In treatment P_w, the probiotic was added directly to the water at the recommended dosage. In