Fish Physiol Biochem (2010) 36:501–509
DOI 10.1007/s10695-009-9320-z

Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response

Xuxia Zhou · Ziqiang Tian · Yanbo Wang · Weifen Li

Received: 28 July 2008 / Accepted: 22 March 2009 / Published online: 11 April 2009

Abstract A feeding trial was conducted for 40 days to delineate the effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. About 360 juveniles were randomly distributed into four treatment groups, each with three replicates. Different probiotics (T-1, *Bacillus subtilis* B10; T-2, *Bacillus coagulans* B16; T-3, *Rhodopseudomonas palustris* G06) were added to the water of tanks at final concentration of 1×10^7 cfu ml^{-1} every 2 days, with no probiotic added to control tanks. At the end of the feeding trial, fish treated with *B. coagulans* B16 (T-2) and *R. palustris* G06 (T-3) had significantly ($P < 0.05$) higher final weight, daily weight gain, and specific growth rate compared with those treated with *B. subtilis* B10 (T-1) and those without probiotics (control). The highest ($P < 0.05$) content of total serum protein was found in T-2 compared with that in T-1, T-3, and the control. However, albumin concentration and albumin/globulin ratio were not affected by the probiotics treatments. Compared with the control, probiotic supplementation remarkably improved activities of superoxide dismutase and catalase ($P < 0.05$). T-2 fish exhibited higher average myeloperoxidase activity than the control, T-1, and T-3 groups. Regarding serum lysozyme content in tilapia, assays showed no difference ($P > 0.05$) among the treatment groups. Furthermore, probiotics treatments remarkably increased respiratory burst activity compared with control, with T-2 showing higher values than T-1 and T-3. This indicated that treatment with probiotics, *B. coagulans* B16 and *R. palustris* G06, as water additives could be used to enhance immune and health status, thereby improving growth performance of *O. niloticus*.

Keywords Probiotic · Tilapia · *Oreochromis niloticus* · Growth performance · Immune response

X. Zhou · W. Li (✉)
Microbiology Division, College of Animal Science, Zhejiang University, 164# Qiutao North Road, 310029 Hangzhou, China
e-mail: wfli@zju.edu.cn

Z. Tian
River and Coastal Environment Research Center, Chinese Research Academy of Environmental Sciences, 100012 Beijing, China

Y. Wang
Food Quality and Safety Department, Zhejiang Gongshang University, 310035 Hangzhou, China

Introduction

With the development of commercial-scale aquaculture, it has become apparent that disease can be a significant limiting factor (Gomez-Gil et al. 2000). Due to the high mortality and contagious nature of disease, large amounts of antibiotics are often used

for therapy. However, indiscriminate use of antibiotics has led to development of drug-resistant bacteria that are becoming increasingly difficult to control and eradicate (Esiobu et al. 2002; Nomoto 2005). Therefore, the need for alternative techniques is increasing and the contribution of probiotics may be considerable.

The term "probiotic" was introduced by Parker (1974). According to his original definition, probiotics are "organisms and substances which contribute to intestinal microbial balance." A growing number of studies have dealt explicitly with probiotics, and it is now possible to survey its state of the art, from empirical use to the scientific approach (Gatesoupe 1999; Wang et al. 2005; Wang and Xu 2006; Vine et al. 2006; Wang 2007; Kesarcodi-Watson et al. 2008). Although probiotics for human and terrestrial animals are dominantly lactic acid bacteria (LAB), many different genera, including photosynthetic bacteria, yeast, and *Bacillus* have been evaluated as probiotics in fish (Gatesoupe 1999; Gunther and Montealegre 2004; El-Haroun et al. 2006; Shelby et al. 2006; Vine et al. 2006; Aly et al. 2008a, b). Moriarty (1998) proposed to extend the definition of probiotics in aquaculture to microbial "water additives." The bacteria of *Bacillus* spp. and *Rhodopseudomonas palustris* had been documented as water-cleansing bacteria (Chen and Chen 2001; Lee et al. 2002; Kim et al. 2005; Satoh et al. 2006; Taoka et al. 2006a, b). Therefore, this study attempted to investigate the effect of probiotics, *Bacillus subtilis* (*B. subtilis*), *Bacillus coagulans* (*B. coagulans*), and *R. palustris* on growth performance, serum biochemical analysis, and immune response of tilapia.

Materials and methods

Probiotics strains

The probiotics, *B. subtilis* B10, *B. coagulans* B16, and *R. palustris* G06 were isolated from a carp (*Cyprinus carpio*) pond in Haining, China. They were cultured in the laboratory and checked routinely for purity based on their morphological and biochemical characteristics during this investigation (Holt 1989). The probiotics strain, *B. subtilis* B10 and *B. coagulans* B16 were cultured and counted on normal nutrient agar by spore staining with the spread plate technique (Austin 1988). *R. palustris* G06 assay cultures were grown on malate basal medium according to Van Niel (1971).

Fish and experimental design

Three hundred and sixty healthy tilapia (*Oreochromis niloticus*) were provided by the Fish Hatchery of Hangzhou, China and randomly allocated to four treatments, each of which had three 200-l tanks of 30 fish per tank. The tilapia received the same diet, containing 37.53% crude protein, 3.82% crude fat, and 10.79% crude ash related to wet matter, and 6.89% moisture, based on NRC (1993) recommendations. Different probiotics (T-1, *Bacillus subtilis* B10; T-2, *Bacillus coagulans* B16; T-3, *Rhodopseudomonas palustris* G06) were added to water of the tanks at final concentration of 1×10^7 cfu ml^{-1} every 2 days, with no probiotic added to control tanks. Three selected strains were grown in each culture medium in a shaking incubator at 30°C. After incubation, the cells were harvested by centrifugation (2,000*g*), washed three times with phosphate buffered saline (PBS, pH 7.2, Sangon, China), and resuspended in the same buffer before use. The trial was carried out for 40 days. The tanks were supplied with running fresh water filtered through a special cotton filter (flow rate 1 l min^{-1}), then passed successively through a tungsten heater. In this study, there was a separate recirculation system for every tank. All fish were maintained at 25 ± 1°C with 50% water change every 2 days before the addition of probiotics and 12 h dark/12 h light photoperiod during the entire trial. For water quality control, temperature and dissolved oxygen were measured daily, and measurements of total ammonium, nitrite, and pH levels were made weekly using the Hach kit model DREL 2400 (Hach Company, CO, USA). Dissolved oxygen level was maintained above 6.0 mg l^{-1} by setting the air pump. Fish were fed two times daily at 08:00 h and 18:00 h with diet. Daily feeding rate was about 3% of total body weight (g) and was properly regulated according to actual intake of tilapia.

Sampling and analytical methods

Weight of all collected tilapia determined at the 1st and 40th day were treated as initial weight and final weight, respectively. Fish survival was also

determined by counting individuals in each tank. Daily weight gain (g day^{-1}) (DWG) was calculated as: (final weight − initial weight)/40 (g day^{-1}). Specific growth rate (SGR) was calculated using the following formula: $100(\ln W_2 - \ln W_1)\ T^{-1}$. Where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days (40) in the feeding period.

At the end of the 40-day culture, fish were sampled randomly from each tank and anaesthetized in diluted MS-222 (ethyl 3-aminobenzoate methanesulfonate, Tricaine; Sigma; 1:2,500) in order to study the effect of probiotics on biochemical indexes and immune responses. Blood from eight fish was withdrawn from caudal vein into plastic Eppendorf tubes using 1-ml syringe and no. 7 needle containing anticoagulant solution. For serum samples preparation, blood was collected into Eppendorf tubes without anticoagulant in syringe and mixed and kept at 4°C for 12 h and then centrifuged (4°C, 3,000*g* for 15 min). The supernatants were collected as the serum samples and stored immediately at −70°C until use.

The serum samples collected earlier were analyzed for total protein using the method of Bradford (1976) and albumin content according to Doumas et al. (1971). The globulin content (subtracting albumin from total protein) and albumin/globulin (A/G) ratio were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Furthermore, superoxide dismutase (SOD) activity, catalase (CAT) activity, and total antioxidation competence (T-AOC) were also evaluated using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) based on the methods described in the instructions.

Assays for myeloperoxidase (MPO) activity in tilapia serum were carried out as described by Kuamari and Sahoo (2005) with slight modification. Serum samples (10 µl) were diluted ten times with Hanks balance saline solution (HBSS, Ca^{2+} and Mg^{2+} free) in 96-well plates, and then freshly prepared substrate of MPO, 35 µl 20 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride (TMB, Sigma), and 5 mM H_2O_2 were added and incubated at 30°C for 30 min to allow the color change reaction to progress completely. Then the color reaction was stopped by adding 35 µl 4 M sulfuric acid (H_2SO_4) and optical density was read at 450 nm by plate reader (Thermo Multiskan MK3). Standard samples without serum were also analyzed. One unit of MPO activity was defined as when 1 µmol H_2O_2 was reduced by each liter of serum at 30°C.

A turbidimetric assay utilizing lyophilized *Micrococcus flavus* NCIMB8166 was used to determine serum lysozyme content according to Parry et al. (1965) with a slight modification. Lyophilized *M. flavus* cells were resuspended in 2.0 ml 0.05 M phosphate buffer (pH 6.2) at concentration of 0.25 mg ml^{-1} and incubated at 30°C for 5 min, after which 200 µl serum was added to 2.0 ml suspension and transmittance was measured after 5 and 125 s at 540 nm (Thermo Multiskan MK3). The lysozyme content was calculated as follows: Lysozyme content =amount of sample causing a decrease in transmittance/the amount of standard causing a decrease in absorbance × concentration of the standard.

Respiratory burst activity of the phagocytes was determined by nitroblue tetrazolium (NBT, Sigma) assay following the method of Choudhury et al. (2005). About 50 µl blood was placed into the wells of 96-well plate and incubated at 30°C for 60 min to allow adhesion of cells. Then the supernatant was removed and the wells were washed three times in PBS (pH 7.2). After washing, 50 µl 0.2% NBT was added and incubated for a further 60 min. The cells were then fixed with 100% methanol for 3 min and washed three times with 30% methanol. The plates were air-dried and 60 µl 2 N potassium hydroxide and 70 µl dimethyl sulfoxide were added to each well. Absorbance (OD) was read at 540 nm in a plate reader (Thermo Multiskan MK3).

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significant variation between the different tested groups. Statistical significance was settled at a probability value of $P < 0.05$. All statistics were performed using SPSS version 11.5 (SPSS, Chicago, IL, USA) for Windows.

Results

Growth performance

There were no significant differences in total ammonium (0.00–0.05 mg l^{-1}), nitrite (0.00–0.01 mg l^{-1}),

and pH (7.0–7.5) of water among all the tanks. The results of growth performance of tilapia (*O. niloticus*) treated with (T-1, T-2, and T-3) or without (control) probiotics are presented in Table 1. In the case of initial weight and survival rate, no significant differences ($P > 0.05$) were observed in the present study. At the end of the feeding trial, fish treated with *B. coagulans* B16 (T-2) and *R. palustris* G06 (T-3) as water additives had significantly ($P < 0.05$) higher final weight, DWG, and SGR compared with those treated with *B. subtilis* B10 (T-1) and without probiotics (control). However, there was no remarkable difference ($P > 0.05$) between T-2 and T-3. No differences were observed for final weight, DWG, and SGR between T-1 and the control.

Biochemical analysis

The effects of probiotics (T-1, *B. subtilis* B10; T-2, *B. coagulans* B16; T-3, *R. palustris* G06) supplemented as water additive on the concentrations of total serum protein, albumin, globulin, and A/G ratio of tilapia (*O. niloticus*) are summarized in Table 2. The highest ($P < 0.05$) content of total serum protein (53.75 ± 3.59 g l^{-1}) was found in T-2 compared with that in T-1 (46.13 ± 2.09 g l^{-1}), T-3 (41.36 ± 5.87 g l^{-1}), and the control (43.16 ± 5.04 g l^{-1}). However, the concentration of total serum protein was not significantly difference among T-1, T-3, and the control ($P > 0.05$). Albumin concentration and A/G ratio in tilapia serum samples were not affected by the probiotics treatments. Compared with the control and T-3, *B. coagulans* B16 supplementation as water additive remarkably improved globulin concentration ($P < 0.05$). Addition of *B. subtilis* B10 in tanks water numerically improved the globulin concentration in fishes compared with T-3 and the control, but the improvement was not significant ($P > 0.05$). Moreover, no significant difference in globulin content of tilapia between T-1 and T-2 was detected.

Immune response

The SOD activity, CAT activity, and T-AOC of tilapia serum samples treated with (T-1, T-2, and T-3) or without (control) probiotics as water additives are shown in Table 3. Compared with the control, probiotic supplementation remarkably improved

Table 1 Growth performance of tilapia (*Oreochromis niloticus*) treated with (T-1, T-2, and T-3) or without (control) probiotics

Group/treatment	Control	T-1	T-2	T-3
Initial weight (g)	6.93 ± 0.51a	7.05 ± 0.42a	6.73 ± 0.46a	6.87 ± 0.45a
Final weight (g)	28.15 ± 2.06a	28.76 ± 1.98a	32.98 ± 1.73b	33.05 ± 1.64b
Survival (%)	100a	100a	100a	100a
DWG (g day^{-1})	0.53 ± 0.04a	0.54 ± 0.04a	0.66 ± 0.03b	0.65 ± 0.03b
SGR (%)	3.50 ± 0.02a	3.51 ± 0.03a	3.97 ± 0.05b	3.93 ± 0.09b

Results relating to growth performance of tilapia including initial weight, final weight, DWG, and SGR are presented as means ± standard error (SE) of triplicate observations. Means in the same row with different lower-case letters are significantly different ($P < 0.05$)

DWG daily weight gain, *SGR* specific growth rate, *T-1 B. subtilis* B10, *T-2 B. coagulans* B16, *T-3 R. palustris* G06

Table 2 Effects of probiotics (T-1, *B. subtilis* B10; T-2, *B. coagulans* B16; T-3, *R. palustris* G06) on the concentrations of total serum protein, albumin, globulin, and A/G ratio of tilapia, *Oreochromis niloticus*

Group/treatment	Control	T-1	T-2	T-3
Total serum protein (g/l)	43.16 ± 5.04a	46.13 ± 2.09a	53.75 ± 3.59b	41.36 ± 5.87a
Albumin (g/l)	3.84 ± 0.47a	3.77 ± 0.29a	3.72 ± 0.21a	3.89 ± 0.19a
Globulin (g/l)	39.29 ± 5.48a	42.36 ± 1.97ab	50.02 ± 3.74b	37.63 ± 4.17a
A/G ratio	0.10 ± 0.01a	0.09 ± 0.00a	0.08 ± 0.01a	0.10 ± 0.01a

Results of tilapia serum biochemical analysis were presented as means ± SE of triplicate observations. Means in the same row with different lower-case letters were significantly different ($P < 0.05$)

A/G ratio albumin/globulin ratio

Table 3 Effects of probiotics (T-1, *B. subtilis* B10; T-2, *B. coagulans* B16; T-3, *R. palustris* G06) on activities of SOD, CAT, and T-AOC of tilapia, *Oreochromis niloticus*

Group/treatment	Control	T-1	T-2	T-3
SOD (U ml^{-1})	48.46 ± 5.04a	97.85 ± 8.86b	86.54 ± 7.13b	81.36 ± 7.13b
CAT (U ml^{-1})	3.98 ± 0.38a	5.63 ± 0.67b	5.72 ± 0.72b	6.03 ± 0.63b
T-AOC (U ml^{-1})	5.64 ± 0.48a	5.79 ± 0.53a	6.05 ± 0.58a	6.19 ± 0.57a

Results were presented as means ± SE of triplicate observations. Means in the same row with different lower-case letters were significantly different ($P < 0.05$)

SOD superoxide dismutase, *CAT* catalase, *T-AOC* total antioxidation competence

activities of SOD and CAT ($P < 0.05$). However, there was no significant difference ($P > 0.05$) between treatments (T-1, T-2, and T-3). Furthermore, serum T-AOC of tilapia was not affected by probiotics treatments.

The effects of probiotics on the myeloperoxidase (MPO) activity are shown in Fig. 1. The results obtained in the present research showed that the groups receiving *B. coagulans* B16 (T-2) exhibited higher average MPO activity (164.67 ± 13.05 U) than the control (73.33 ± 6.11 U), T-1 (86.67 ± 7.51 U), and T-3 (96.34 ± 10.07 U) groups after 40 days of treatment. MPO activity was not significantly different between groups receiving the probiotics *B. subtilis* B10 (T-1) and *R. palustris* G06 (T-3). Furthermore, no remarkable difference ($P > 0.05$) was observed in MPO activity between T-1 and the control. For serum lysozyme content in tilapia, assays showed no difference ($P > 0.05$) among all treatment groups (Fig. 2). The data on respiratory burst

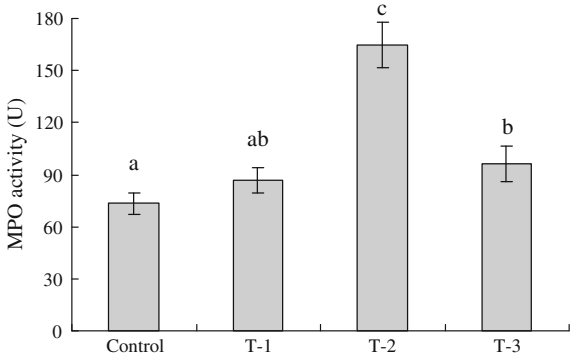

Fig. 1 Myeloperoxidase (MPO) activity of tilapia treated with probiotics, *B. subtilis* B10 (T-1), *B. coagulans* B16 (T-2), and *R. palustris* G06 (T-3), and without probiotics (control) at the end of 40 days culture. Means with different superscripts were significantly different ($P < 0.05$)

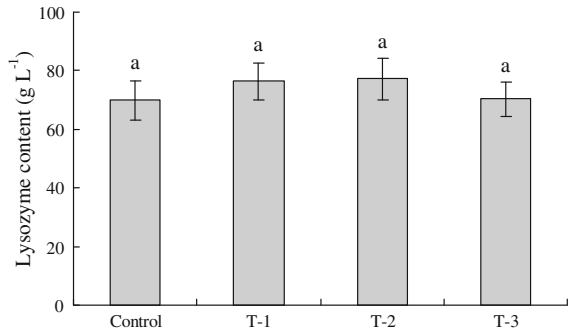

Fig. 2 Serum lysozyme content of tilapia treated with probiotics, *B. subtilis* B10 (T-1), *B. coagulans* B16 (T-2), and *R. palustris* G06 (T-3), and without probiotics (control) at the end of 40 days of culture. Means with different lower-case letters are significantly different ($P < 0.05$)

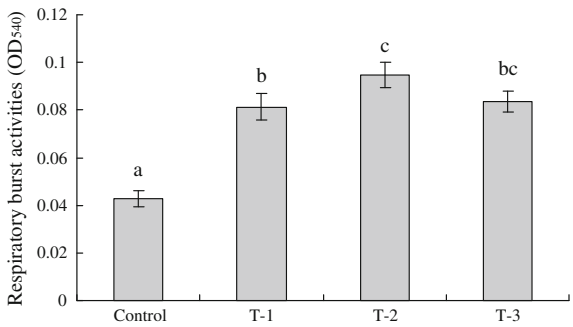

Fig. 3 Respiratory burst activities (OD_{540}) of tilapia treated with probiotics, *B. subtilis* B10 (T-1), *B. coagulans* B16 (T-2), and *R. palustris* G06 (T-3), and without probiotics (control) at the end of 40 days of culture. Means with different lower-case letters were significantly different ($P < 0.05$)

activities (OD_{540}) of tilapia treated with and without probiotics at the end of 40 days culture are shown in Fig. 3. In our study, a significant increase ($P < 0.05$) in fish serum activity of respiratory burst was observed in the groups treated with probiotics

compared with the control without any probiotic. Respiratory burst activity in T-2 was remarkably higher ($P < 0.05$) than that in T-1. However, there was no significant difference ($P > 0.05$) between T-2 and T-3. In addition, also no significant difference was observed in T-1 and T-3.

Discussion

Application of bacteria for their potential benefit as probiotics in aquaculture is increasing (Balcázar et al. 2006; Gatesoupe 2007). Aly et al. (2008b) evaluated the potential benefit of *Bacillus pumilus* as a probiotic in the culture of tilapia (*O. niloticus*) and showed that a low dose of *Bacillus pumilus* (10^6 g^{-1} diet fed) induced significant increases in weight gain when used for 2 months. A similar finding was obtained by Taoka et al. (2006a), who investigated the effect of commercial probiotics (containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *Saccharomyces cerevisiae*) on growth of Japanese flounder (*Paralichthys olivaceus*) and showed that addition of probiotics to diet or rearing water resulted in enhanced growth of the flounder. In this work, it was clear that administration of probiotics, *B. coagulans* B16 and *R. palustris* G06 via tank water, had beneficial effects on growth performance of tilapia, *O. niloticus*. Overall, the beneficial effects of probiotics on fish performance parameters were in agreement with a larger number of other researchers who studied the use of probiotics in tilapia (Lara-Flores et al. 2003; El-Haroun et al. 2006; Wang et al. 2008) compared with studies lacking positive effects (Gunther and Montealegre 2004; Shelby et al. 2006). However, there was no significant difference in growth performance of T-1 treated with *B. subtilis* B10 compared with the control. This indicated that the kind of probiotic used was one of the factors promoting growth performance of tilapia. In addition, there were no significant effects of probiotics on water quality, such as total ammonium, nitrite or pH, among all groups; they were stable and within acceptable ranges (Boyd and Tucker 1998).

In the present research, fish treated with *B. coagulans* B16 as water additive showed higher concentrations of serum protein and globulin than the control. This was in agreement with previous studies which showed that serum protein and globulin in fish treated with different immunostimulants, i.e., β-glucan and yeast RNA, were always higher than the control (Choudhury et al. 2005; Misra et al. 2006). However, compared with the control, no significant difference in serum protein and globulin contents of tilapia treated with *B. subtilis* B10 (T-1) and *R. palustris* G06 (T-3) was detected. Similar result was obtained by Wang et al. (2008), who investigated the effect of a probiotic bacterium, *Enterococcus faecium* ZJ4, on growth performances of tilapia (*O. niloticus*) and showed that the bacterium did not affect the concentration of serum protein and globulin contents. Therefore, it was necessary to consider the possibility of strain differences, as suggested by Bogut et al. (1998) and Lara-Flores et al. (2003).

Amongst the modes of action through which probiotic bacteria exerted their beneficial effects, there is increasing evidence that such bacteria could also effectively improve host innate and adaptive immune responses (Austin et al. 1995; Isolauri et al. 2002; Heyman and Ménard 2002; Taoka et al. 2006a, b). In our study, the presence of probiotics, *B. coagulans* B16 and *R. palustris* G06, indicated that they were able to increase immune responses such as MPO activity, respiratory burst activities, SOD activity, and CAT of tilapia. These results suggest that increased growth performance might be attributed to improved immune responses. On the contrary, no specific immune enhancement was observed in young tilapia, *O. niloticus*, treated with commercial probiotics (Shelby et al. 2006). However, this does not preclude the usefulness of probiotics in aquaculture because the commercial probiotics used were developed for terrestrial livestock and might be unsuitable for tilapia.

The activities of MPO were higher in the T-2 and T-3 than in the control group. Overall, the beneficial effects of probiotics on MPO activity were in agreement with a larger number of other researchers who studied use of probiotics in aquaculture (Rodríguez et al. 2004; Kumari and Sahoo 2006; Wang et al. 2008). This indicates that modulation of MPO activity was one of the most immediate and key effects produced by probiotic bacteria on the host immune system.

The level of serum lysozyme was thought to be mainly due to phagocytic cells, monocytes, and neutrophils (Murray and Fletcher 1976). In the

present study, serum lysozyme content of tilapia was not affected by treatment with probiotics as water additives. On the other hand, it appeared that this finding was responsible for the 100% survival after 40 days of culture in the present study. The previous study showed that serum lysozyme activities of tilapia decreased after *Edwardsiella tarda* pathogenic infection in all groups and that the decrease was greater in fish treated with probiotics (Pirarat et al. 2006). This might be associated with our experimental conditions, which maintained good tank water quality and assured no pathogenic infection during 40 days of culture. However, it had been shown that injection of ß-glucan induced significantly elevated lysozyme activity (Misra et al. 2006). A similar finding was obtained by Taoka et al. (2006a, b), who investigated the effect of commercial probiotic on the nonspecific immune system of tilapia (*O. niloticus*) and showed that addition of probiotics to the rearing water resulted in enhanced lysozyme activity. Thus, further studies are needed to reveal the relationship between lysozyme activity fluctuations and different immunostimulants such as some pro- and prebiotics.

The NBT test used to determine respiratory burst activity showed significantly increased values in all the current tested groups that were given probiotics when compared with the control group. The groups given *B. coagulans* B16 showed higher values than the other groups, suggesting that the probiotics might enhance nonspecific immune responses. Salinas et al. (2006) reported that respiratory burst activity of teleost fish (*Sparus aurata* L.) increased in vitro by the addition of heat-inactivated *Lactobacillus delbrüeckii* ssp. *Lactis*. Similar result was also observed by Nikoskelainen et al. (2003), who reported that rainbow trout fed *L. rhamnosus* (8×10^4 cfu g^{-1}) for 2 weeks showed a significant increase in respiratory burst activity compared with the control group. Further studies of respiratory burst activity are needed to better understand the exact reason why different probiotics strains supplemented in tanks as water additives show different results.

It could be concluded that treatment with probiotics, *B. coagulans* B16 and *R. palustris* G06, as water additives could be used to enhance immune and health status, thereby improving growth performance of *O. niloticus*. However, further extensive testing, including combined probiotics effects and full commercial cost–benefit analysis, is necessary before recommending its widespread application in aquaculture. The addition of probiotics to aquaculture, in general, requires further research to clearly understand the functional mechanism among the microorganisms, and how probiotics work in the digestive tract of tilapia or other fish.

Acknowledgements This study was supported by the fund of the National Natural Science Foundation of China and National Basic Research Program of China (973 Program) (No. 2009 CB118705). Thanks are due to all workers involved.

References

Aly SM, Abd-El-Rahman AM, John G, Mohamed MF (2008a) Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. Aquaculture 277:1–6. doi:10.1016/j.aquaculture.2008.02.021

Aly SM, Mohamed MF, John G (2008b) Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). Aquac Res 39:647–656. doi:10.1111/j.1365-2109.2008.01932.x

Austin B (1988) Methods in aquatic bacteriology. Wiley, New York

Austin B, Stuckey LF, Robertson PAW, Effendi I, Griffith DRW (1995) A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio ordalii*. J Fish Dis 18:93–96. doi:10.1111/j.1365-2761.1995.tb01271.x

Balcázar JL, de Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Múzquiz JL (2006) The role of probiotics in aquaculture. Vet Microbiol 114:173–186. doi:10.1016/j.vetmic.2006.01.009

Bogut I, Milakovic Z, Bukvic Z, Brkic S, Zimmer R (1998) Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). Czech. J Anim Sci 439:231–235

Boyd CE, Tucker CS (1998) Pond aquaculture water quality management. Kluwer Academic Publishers, USA

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein. Anal Biochem 72:248. doi:10.1016/0003-2697(76)90527-3

Chen CC, Chen SN (2001) Water quality management with *Bacillus* spp. in the high-density culture of red-parrot fish *Cichlasomia citrinellum* × *C-synspilum*. N Am J Aquac 63:66–73. doi:10.1577/1548-8454(2001)063<0066:WQMWBS>2.0.CO;2

Choudhury D, Pal AK, Sahu NP, Kumar S, Das SS, Mukherjee SC (2005) Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juiveniles. Fish Shellfish Immunol 19:281–291. doi:10.1016/j.fsi.2005.01.004

Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocersol green. Clin Chim Acta 31:87–96. doi:10.1016/0009-8981(71)90365-2

El-Haroun ER, Goda AMAS, Chowdhury KMA (2006) Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquac Res 37:1473–1480. doi:10.1111/j.1365-2109.2006.01584.x

Esiobu N, Armenta L, Ike J (2002) Antibiotic resistance in soil and water environments. Int J Environ Health Res 12:133–144. doi:10.1080/09603120220129292

Gatesoupe FJ (1999) The use of probiotics in aquaculture. Aquaculture 180:147–165. doi:10.1016/S0044-8486(99)00187-8

Gatesoupe FJ (2007) Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture 267:20–30. doi:10.1016/j.aquaculture.2007.01.005

Gomez-Gil B, Roque A, Turnbull JF (2000) The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 191:259–270. doi:10.1016/S0044-8486(00)00431-2

Gunther J, Montealegre RJ (2004) Effect of the probiotic *Bacillus subtilis* on the growth and food utilization of tilapia (*Oreochromis niloticus*) and prawn (*Macrobrachium rosenbergii*) under laboratory conditions. Rev Biol Trop 52:937–943

Heyman M, Ménard S (2002) Probiotic microorganisms: how they affect intestinal pathophysiology. Cell Mol Life Sci 59:1–15. doi:10.1007/s00018-002-8494-7

Holt JG (1989) Bergey's manual of systematic bacteriology (*Archaeobacteria, Cyanobacteria*, and remaining gram-negative *Bacteria*), vol 3, 1st edn. Williams and Wilkins, Baltimore, MD

Isolauri E, Kirjavainen PV, Salminen S (2002) Probiotics: a role in the treatment of intestinal infection and inflammation. Gut 50:54–59. doi:10.1136/gut.50.suppl_3.iii54

Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274:1–14. doi:10.1016/j.aquaculture.2007.11.019

Kim JK, Park KJ, Cho KS, Nam SW, Park TJ, Bajpai R (2005) Aerobic nitrification denitrification by heterotrophic *Bacillus* strains. Bioresour Technol 96:1897–1906. doi:10.1016/j.biortech.2005.01.040

Kuamari J, Sahoo PK (2005) Effects of cyclophosphamide on the immune system and disease resistance of Asian catfish *Clarias batrachus*. Fish Shellfish Immunol 19:307–316. doi:10.1016/j.fsi.2005.01.008

Kumari J, Sahoo PK (2006) Dietary β-1,3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L.). Fish Dis. 29:95–101. doi:10.1111/j.1365-2761.2006.00691.x

Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BE, López-Madrid W (2003) Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216:193–210. doi:10.1016/S0044-8486(02)00277-6

Lee DY, Ramos A, Macomber L, Shapleigh JP (2002) Taxis response of various denitrifying bacteria to nitrate and nitrite. Appl Environ Microbiol 68:2140–2147. doi:10.1128/AEM.68.5.2140-2147.2002

Misra CK, Das BK, Mukherjee SC, Pattnaik P (2006) Effect of multiple injections of β-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish Shellfish Immunol 20:305–319. doi:10.1016/j.fsi.2005.05.007

Moriarty DJW (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164:351–358. doi:10.1016/S0044-8486(98)00199-9

Murray CK, Fletcher TC (1976) The immunohistochemical localization of lysozyme in plaice (*Pleuronectes platessa* L.) tissues. J Fish Biol 9:329–334. doi:10.1111/j.1095-8649.1976.tb04681.x

National Research Council (NRC) (1993) Nutrient requirements of fish. National Academy Press, Washington, DC, p 114

Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius EM (2003) Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish Shellfish Immunol 15:443–452. doi:10.1016/S1050-4648(03)00023-8

Nomoto K (2005) Prevention of infections by probiotics. J Biosci Bioeng 100:583–592. doi:10.1263/jbb.100.583

Parker RB (1974) Probiotics, the other half of the antibiotics story. Anim Nutr Health 29:4–8

Parry RM, Chandan RC, Shahani KM (1965) A rapid and sensitive assay of muramidase. Proc Soc Exp Biol NY 119:384–386

Pirarat N, Kobayashi T, Katagiri T, Maita M, Endo M (2006) Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*). Vet Immunol Immunopathol 113:339–347. doi:10.1016/j.vetimm.2006.06.003

Rodríguez A, Cuesta A, Esteban MA, Meseguer J (2004) The effect of dietary administration of the fungus *Mucor circinelloides* on non-specific immune responses of gilthead seabream. Fish Shellfish Immunol 16:241–249. doi:10.1016/S1050-4648(03)00082-2

Salinas I, Diaz-Rosales P, Cuesta A (2006) Effect of heat-inactivated fish and non-fish derived probiotics on the innate immune parameters of a teleost fish (*Sparus aurata* L.). Vet Immunol Immunopathol 111:279–286. doi:10.1016/j.vetimm.2006.01.020

Satoh H, Yamakawa T, Kindaichi T, Ito T, Okabe S (2006) Community structures and activities of nitrifying and denitrifying bacteria in industrial wastewater-treating biofilms. Biotechnol Bioeng 94:762–772. doi:10.1002/bit.20894

Shelby R, Lim C, Yildirim-Aksoy M, Delaney MA (2006) Effects of probiotic diet supplements on disease resistance and immune response of young Nile Tilapia, *Oreochromis niloticus*. J Appl Aquac 18:49–60. doi:10.1300/J028v18n02_02

Taoka Y, Maeda H, Jo JY, Jeon MJ, Bai SC, Lee WJ, Yuge K, Koshio S (2006a) Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fish Sci 72:310–321. doi:10.1111/j.1444-2906.2006.01152.x

Taoka Y, Maeda H, Jo JY, Kim SM, Park SI, Yoshikawa T, Sakata T (2006b) Use of live and dead probiotic cells in

tilapia *Oreochromis niloticus*. Fish Sci 72:755–766. doi:10.1111/j.1444-2906.2006.01215.x

Van Niel CB (1971) Techniques for the enrichment, isolation and maintenance of the photosynthetic bacteria. In: San Pietro A (ed) Methods in enzymology. London, Academic Press, pp 3–28

Vine NG, Leukes WD, Kaiser H (2006) Probiotics in marine larviculture. FEMS Microbiol Rev 30:404–427. doi:10.1111/j.1574-6976.2006.00017.x

Wang YB (2007) Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 269:259–264. doi:10.1016/j.aquaculture.2007.05.035

Wang YB, Xu ZR (2006) Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim Feed Sci Technol 127:283–292. doi:10.1016/j.anifeedsci.2005.09.003

Wang YB, Xu ZR, Xia MS (2005) The effectiveness of commercial probiotics in Northern White Shrimp (*Penaeus vannamei* L.) ponds. Fish Sci 71:1034–1039

Wang YB, Tian ZQ, Yao JT, Li WF (2008) Effect of probiotics, *Enteroccus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. Aquaculture 277:203–207. doi:10.1016/j.aquaculture.2008.03.007