Turkish Journal of Fisheries and Aquatic Sciences 8: 43-48 (2008)

Growth, Survival and Gut Microbial Load of Rainbow Trout (*Onchorhynchus mykiss*) Fry Given Diet Supplemented with Probiotic during the Two Months of First Feeding

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Received 11 June 2007
Accepted 03 January 2008

Abstract

A commercial *Bacillus* spp. probiotic was tested on rainbow trout fry during the two months of first feeding. Probiotic was introduced in diets at five different levels, (T_1: 4.8×10^8, T_2: 1.2×10^9, T_3: 2.01×10^9, T_4: 3.8×10^9, T_5: 6.1×10^9 CFU g^{-1}) and their effects compared with those of control diet containing no probiotic. Survival in treatments was significantly ($P<0.05$) higher than control and a slight increasing mortality rate was observed during the first week of experiment. The counts of bacteria associated with trout intestine in all treatments were significantly ($P<0.05$) higher than controls and *Bacillus* spp. was not detected in controls. Total bacteria counts were significantly different among treatments and controls; it may suggest that the colonization rate of digestive tracts of rainbow trout fry with bacteria was affected by dietary bacteria level. Specific growth rate, condition factor, protein efficiency ratio were slightly but significantly ($P<0.05$) higher and feed conversion ratio was lower in groups received probiotic via diets than controls. It may show that probiotic stimulates digestive development and enzymatic activity in fish. Growth performance in treatment received 3.8×10^9 CFU g^{-1} showed the best results. Therefore, it does not appear that higher levels of probiotics improved results and suitable doze of probiotic should be assessed before application in large scale to prevent any undesired effects. The supplementation of trout starter diet with *Bacillus* spp. is probably effective for improving rearing conditions.

Key words: *Bacillus*, micro flora, nutrition.

Introduction

Rainbow trout culture is economically important in Iran and bacterial infectious disease in trout farming seems to be the major reason for decreasing the production level in some farms. Success and failure of fish culture programs are determined by early life stage conditions (Ghosh *et al*., 2002). On the other hand, the occurrence of sub-clinical infections under farming conditions probably lead into reduced growth and increased mortality (Kapetanovic *et al*., 2005). In order to make cessation or reduction on such as undesired results in fry rearing, many feed additives have been used for improving health conditions and the feed utilization efficiency (Ahilan *et al*., 2004). Antibiotics, one of the feed additives, were commonly used in the early 1950s (Ahilan *et al*., 2004). Due to abuse of antibiotics in animal growth promoters, antibiotic resistance has become a common characteristic in microorganisms (Austin *et al*., 1994; Robred *et al*., 2000), thus caused serious problems in microbial infectious treatments (Saarela *et al*., 2000). The use of probiotics, which are beneficial microorganisms or their products with the benefit effects to the hosts, have been used in aquaculture in order to control disease, as supplements for improving growth and in some cases as a mean of replacing antimicrobial compounds (Irianto and Austin, 2002).

Many researches on probiotic for aquaculture have been done (Moriarty, 1998; 1999; Ghosh *et al*., 2002; Paniggraahi *et al*., 2004; Ahilan *et al*., 2004; Salinas *et al*., 2005). Some of the probable modes of action for probiotics include competitive exclusion, i.e. the probiotics inhibit the colonization of potential pathogens in the digestive tract by production of inhibitory compounds or through competition for nutrients and/or space, modification of microbial metabolism, and/or by the stimulation of host immunity responses. What is more, probiotics may produce vitamins and detoxify the compounds in the diets or break down the indigestible compounds, which may lead into the nutritional improvement and stimulate appetite (Irianto and Austin, 2002).

Some works have been done to evaluate competitive exclusion of potential probiotics on rainbow trout (Nikoskelinen, 2002; Ji-Woong, 2003; Irianto and Austin, 2003; Ibrahim *et al*., 2004). Stimulation of immune system in rainbow trout with several candidate probiotics has also been evaluated by some researchers (Irianto and Austin, 2002; Nikoskelainen, 2002; Paniggraahi *et al*., 2004; Ji-Woong, 2003; Kim and Austin, 2006).

Since the *Bacillus* genus has not been reported as pathogens of the aquatic organisms (Moriary, 1998), its application has been promoted and more widely accepted within the aquaculture industry (Gullian *et al*., 2004). *Bacillus* species are able to produce antibiotics, amino acids and enzymes (Sanders *et al*., 2003). Consequently, *Bacillus*

probiotics may have positive nutritional effects on fish.

In the present study, we investigated the effect of *Bacillus* probiotic by administering via diets on digestibility of food, growth and survival of rainbow trout fry, with attention to fry intestinal micro-biota exposed to probiotic, compared to not receiving probiotic.

Material and Methods

Rearing Conditions and Experimental Design

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) (average weight = 120 mg) were obtained from a well–known hatchery in Iran, and maintained in 18 Californian flow-through with continuous fresh water supplied from spring (temperature = 13.98±0.06, mean±standard error) for a period of 63 days. Five treatments were conducted to evaluate the effect of probiotics administered to the rainbow trout fry, each treatments, in triplicate, was stocked with 500 fish. The fish were fed at five different levels, (T_1: 4.8×10^8, T_2: 1.2×10^9, T_3: 2.01×10^9, T_4: 3.8×10^9, T_5: 6.1×10^9 CFU g^{-1}) and control diet groups served as well. The sampling for nutritional effects was carried out once in a week.

Feeding and Probiotic Supplement Preparation

Commercial rainbow trout starter food (SFT0, Chine Co., Tehran) was taken as a basal diet for the supplementation of probiotic. The commercial probiotic used in this experiment (Bio plus 2B, Razak Co., Iran) contained spores of two species of *Bacillus* (i.e., *B. subtilis* and *B. licheniformis*). Probiotics prepared as described in its original manual. The proper amounts of probiotic suspension were sprayed into the feed slowly, mixing part by part in a mixer. Then, the feed was air dried under sterile conditions for 12 h and stored at 20°C. The commercial feed sprayed with sterilized diluents alone served as the control diet.

Fish Dissection and Microbiology

Six fish were collected from each feed trial, after 20 h of starvation at the end of the experiment (Wache *et al*., 2006). After killing the fish, intestines were dissected out in sterile conditions, and then three intestine samples from each treatment were used for microbiological examination and kept in ice until transferring to microbiology laboratory. In the lab, each intestine sample was homogenized and serially diluted with sterilized normal saline solution, and then samples were placed onto tryptic soy agar plates for isolation and total counts of bacteria. The plates were incubated for 27 h at 37°C. After that, for obtaining pure culture, from each sample 20-25 colonies per plate were picked randomly and re–streaked onto nutrient agar plates three times according to the methods of Spangaard *et al*. (2000) and Kapetanovic *et al*. (2005). Observation and identification of all the purified isolated were done according to the cell morphology, motility, gram staining, oxidize and catalas activities followed by Barrow and Feltman (1993) key identification.

Determination of Nutritional Effects and Survival

Every week, 10 samples from each treatment were taken to determine wet weight and total length. The numbers of survivors were recorded as well. At the end of the experiment, carcass of 3 fish, intestines of which were dissected out, was transferred for biochemical analysis. Feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were expressed as following:

$$\mathrm{FCR} = \frac{\text{Dry weight of ingested food}}{\text{Wet weight of produced trout}}$$

$$\mathrm{PER} = \frac{\text{Wet weight of produced trout} \times 100}{\text{Dry weight of ingested protein}}$$

$$\mathrm{SGR} = \frac{(\mathrm{Ln}\, W_t - \mathrm{Ln}\, W_0) \times 100}{T}$$

Where t is the period of culture in days, ln W_0 is the natural logarithm of the weight of the fry at the beginning of the experiment, and ln W_t is the natural logarithm of the weight of the fry at day t. (W_0 and W_t are in gram).

Statistical Analysis

One way analysis of variance (ANOVA; SPSS, 10.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by LSD test. All tests used a significance level of $P<0.05$. Data are reported as means ± standard errors.

Results

Survival and Growth

Under five levels of probiotic supplement, among the experimental fish normal behaviour exhibited and no cannibalism were observed. Probiotic feed acceptance in all treatments was as good as control group. Although mortality was low in both controls and treatments, administering probiotic showed significantly higher survival in all treatments, except T_1 (Figure 1). Higher mean weight and total length were recorded in fish fed with probiotic supplement. Although mean weight just in T_4 was significantly higher than control, total length was not significantly affected by the administered probiotic

(Figure 1). In all probiotic treatments, except T_1, food conversion ratio (FCR) and protein efficiency ratio (PER) were significantly higher than in controls (Figure 2). Specific growth ratio (SGR) of the treated fish was also determined and showed significantly higher rate just in T_4 and T_5 (Figure 2). At the end of the study, the best results were retrieved from T_4. In order to determine the nutritional effects of administered probiotic on rainbow trout fry, the biochemical composition of carcass was analyzed. The results are represented in Table 1. Protein values of carcass in all treatments, except T_1, were significantly higher than controls. The best result was obtained from T_4. Significantly different fat values of carcass in probiotic groups, compared to the controls, were indicated. Moisture values of T_3, T_4 and T_5 indicated a significant difference as well (Table 1).

Intestinal Microbiota

Bacillus was dominant bacteria in probiotic treatments. In contrast, no *Bacillus* strains were detected in control groups (Table 2). Total bacterial counts in trout intestine, which received probiotic, were not significantly different. No clear effects on intestine bacteria associated with different levels of probiotic in treatments and control groups were detected (Figure 3). Vibrionaceae was dominant bacterium in all treatment as well as control groups.

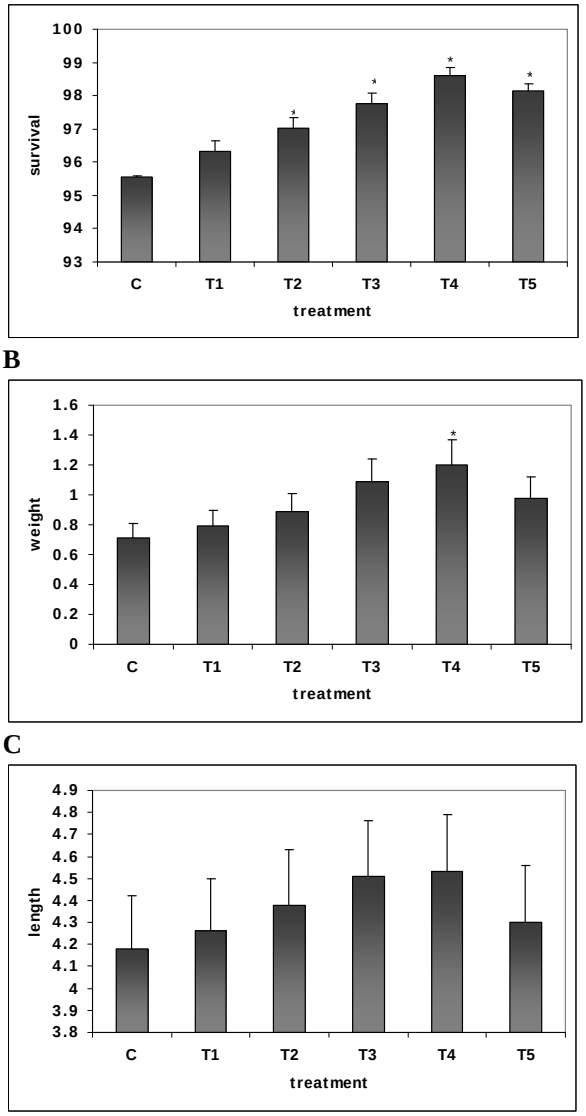

Figure 1. Survival percent (A); Mean weight (B) and Total length (C) of rainbow trout fry in treatments.
C-control, T_1 to T_5-treatments 1 to 5.
* Significantly ($P<0.05$) different.

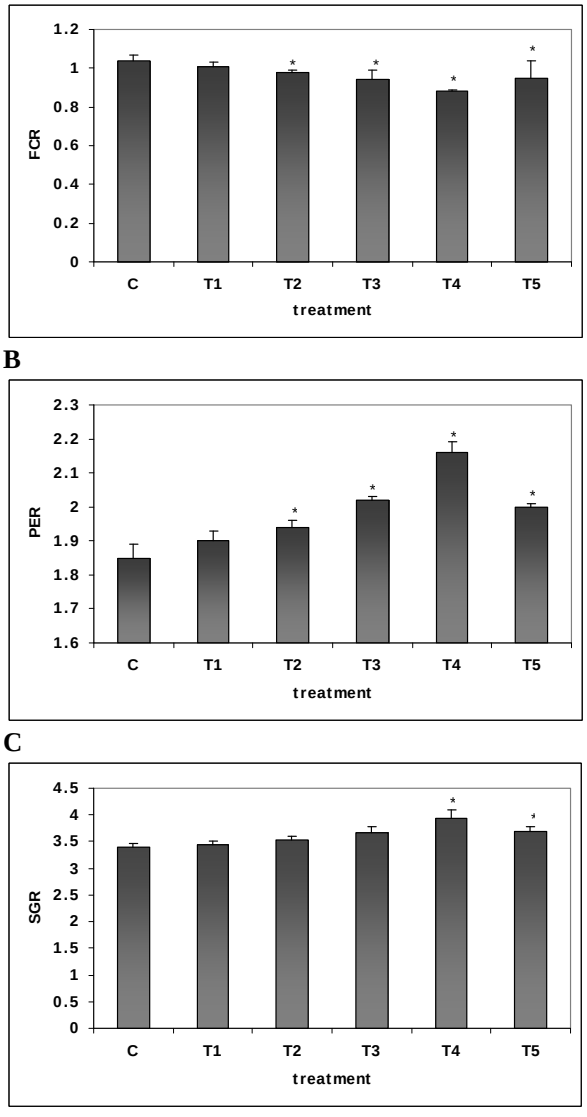

Figure 2. Food convertion ratio (A); Protein efficiency ratio (B) and Specific growth rate (C).
C-control, T_1 to T_5-treatment 1 to 5.
* Significantly ($P<0.05$) different.

Table 1. Chemical composition of carcass after feeding with probiotic

Treatment	Moisture	Fat	Protein
C	74.7±1.4	10.1±0.2*	10.3±0.1
T_1	71.9±0.5	9.0±0.1*	10.8±0.2
T_2	73.4±0.4	8.9±0.1*	11.9±0.1*
T_3	69.9±0.1*	7.8±0.1*	13.7±0.3*
T_4	67.8±0.7*	6.5±0.1*	15.0±0.3*
T_5	69.98±0.5*	5.9±0.0*	12.1±0.3*

Mean±S.E. (N=3). C-control, T_1 to T_5-treatment 1 to 5. * Significantly ($P<0.05$) different.

Table 2. Total bacterial counts and Bacillus in the intestine of rainbow trout fry after feeding for 63 days

Treatment	Mean number of cells g^{-1} (CFU) in intestine 63 days after feeding		
	Probiotic cells	Total microflora	Ratio (%)(probiotic/total
C	—	$12.5±1.08×10^4$	—
T_1	$29.4±2.17×10^6$	$45.2±4.07×10^6$	65.0±2.3
T_2	$11.9±1.28×10^6$	$1.40±0.79×10^6$	85.1±2.0
T_3	$2.52±2.1×10^6$	$2.71±2.90×10^6$	92.9±4.6
T_4	$1.79±1.51×10^7$	$1.82±1.32×10^7$	98.3±4.0
T_5	$3.37±2.05×10^7$	$3.39±2.06×10^7$	99.4±3.8

Mean±S.E. (N=3). C-control, T_1 to T_5-treatment 1 to 5.

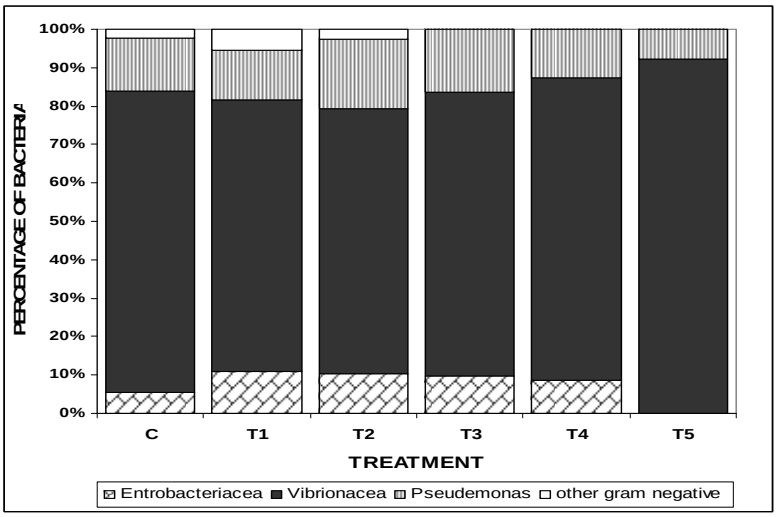

Figure 3. Proportion of dominant group of bacteria characterized in rainbow trout fry.

Discussion

The relative proportion of *Bacillus* spp. in the intestinal flora of rainbow trout fry fed diets containing probiotic, increased with greater density of probiotic supplement in treatments. According to Ringo *et al.* (1995), high proportion is probably related to an increase in suitable attachment sites as a result of histological and functional development of fry and improved internal environmental conditions for bacterial growth (Vine *et al*., 2006). In contrast to constant habitat of terrestrial animals and resident flora in their gastrointestinal tract in aquatic animals, most microbes are transient (Panigrahi *et al*., 2004) and affected by conditions of surrounding water (Gomez-Gill *et al*., 2000). Since fish are poikilotherm animals, one of the primary factors influencing the microbiota of fish perhaps is temperature changes (Panigrahi *et al*., 2004). High proportion of *Bacillus* spp. in the intestinal of experimental fish may shows that intestinal environment is suitable for the given probiotic to settle and grow and also lead into harbour a great number of microbial cells of host intestine. Increase in survival associated with *Bacillus* probiotic proportion in the gut flora is probably due to competitive exclusion of other bacteria, especially with potentially pathogenic bacteria. The identified bacteria of fry intestine were recognized as fish

pathogenic species (Kapetanovic *et al*., 2005). What's more, Enterobacteriaceae population, one of the identified bacteria, in T_5 disappeared and the population of the other bacteria in probiotic treatments declined. It can strongly confirm the idea of out-competing the other bacteria by colonization of probiotic in intestine. On the other hand, survival in T_4 was higher, so we can not definitely conclude that the exclusion of other bacteria by the probiont results in improved survival. However, this effect should not be ignored.

Because growth rate throughout the experiment was improved in T_4, not in T_5, it can be certainly suggested that the more probiotic cells in diets and host intestine necessarily does not result in the more improved growth and survival. Better growth, as observed in T4, may establish better health conditions in rainbow trout fry and therefore, decrease mortality. *Bacillus* spp. produces several peptide antibiotics, including subtilin and bacitracin produced by *B. subtilis* and *B. leicheniformis*, respectively, which was present in the probiotic we used. Moreover, there are a number of other substances with biocontrol activities isolated from species of *Bacillus* (Rosvitz *et al*., 1998). Iturins, cyclic lipoproteins isolated from *B. subtilis* are toxic to a wide range of fungi and yeast (Maget-Dana and Peypoux, 1994). Therefore, administered *Bacillus* gave rise to the fry resistance to pathogens and enhanced survival by producing inhibitory substances to other microorganisms. The *Bacillus* species produce proteases (for example, subtilin), which helps in digestion (Sanders *et al*., 2003). They are also said to produce vitamin K and B_{12} (Rosvitz *et al*., 1998). Gram-positive bacteria, including members of the genus *Bacillus*, secret a wide range of exoenzymes (Moriarty, 1998), which might have supplied digestive enzymes and certain essential nutrients to promote better growth. *Bacillus subtilis* and *B. leicheniformis* can break down proteins and carbohydrates (Rosvitz *et al*., 1998; Farzanfar, 2006). So it can be suggested that administration of *Bacillus* bacteria to trout fry results in enhanced digestion of food and improved growth, including low food conversion ratio (FCR), and high specific growth rate (SGR). High protein efficiency ratio (PER) as well as greater protein values of carcass in probiotic treatments may be due to proteins secreted by members of genus *Bacillus* (Rosvitz *et al*., 1998).

We found that supplementation of trout starter diet with the proper density of commercial *Bacillus* probiotic could be beneficial for growth and survival of rainbow trout fry, especially in fast growing conditions, where it would be essential to stimulate the precocious maturation of digestive system (Wache *et al*., 2006). No clear effect of probiotic on diversity of rainbow trout fry intestine flora detected, but high rate of probiotic bacteria colonization was observed. Since the results might be affected by the rearing conditions (Spanggaard *et al*., 2000), so we suggest the effects of *Bacillus* probiotic to be tested in other locations.

Acknowledgments

We would like to thank the Ghezelpark Trout hatchery and Cold Water Fishes Research Center of Iran for providing the fry and facilities for the study.

References

Ahilan, B., Shine, G. and Santhanam, R. 2004. Influence of probiotics on the growth and gut microflora load of juvenile Gold fish (*Carassius auratus*). Asian Fisheries Science, 17: 271–278.

Ali, A. 2000. Probiotics in fish farming-evaluation of a candidate bacteria mixture. PhD thesis. Uppsala: Swedish University of Agriculture Science.

Anuaud, S. 2000. What is a lactic Acid Bacteria? Functional Foods Journal, 12: 11–20.

Arslan, C. 2004. Effect of dietary probiotic supplementation on growth performance in the Rock Partridge (*Alectoris graeca*). Turk. J. Anim. Sci., 28: 887–891.

Bogut, I., Milakovic, Z., Bukvic, Z., Brkic, S. and Zimmer, R. 1998. Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in Carp (*Cyprinus carpio*). Journal of Animal science, 43: 231-235.

DeSch, R. and Ollevier, F. 2000. Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*. Aquaculture, 186: 107-116.

Eliasson, A. 2003. The physiological characterization of *Bacilus polymyxa*. Post Doc. Project. Biotechnology Institute kolding .

Fuller, R. 1987. A review: probiotics in man and animals. Journal of App. Bacteriology, 66: 365-378.

Galindo, A.B. 2004. *Lactobacillus plantarum* 44A as a live feed supplement for freshwater fish. PhD. thesis. Wageningen: Wageningen University.

Gatesoupe, F.J. 2000. The use of probiotics in aquaculture: A review. Aquaculture, 180: 147–165.

Ghosh, K., Sen, S.K. and Ray, A.K. 2002. Growth and survival of Rohu, *Labeo rohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora. Acta. Icthol. Piscat., 32: 83-92.

Gilberg, A., Johansen, A. and Bogwald, J. 1995. Growth and survival of Atlantic Salmon (*Salmo salar*) fry given diet supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. Aquaculture, 138: 23-34.

Gilberg, A., Mikkelsen, H., Sandaker, E. and Ringo, E. 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). Hydrobiologia, 352: 279-265.

Gomez-Gil, B., Rogue, A. and Turnbull, J.F. 2000. The use and selection of aquatic organisms. Aquaculture, 191: 259-270.

Gram, L., Lovold, T., Melchiorsen, J. and Spanggaard, B. 2001. *In vitro* antagonism of probiont *Pesudomonas fluorescence* strain AH2 against *Aeromonas salmonicida* does not confer protection of salmon against furunculosis. Aquaculture, 199: 1-11.

Gullian, M., Thompson, F. and Rodriguez, J. 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture, 233: 1-14.

Hansen, G.H. and Olfasen, J.A. 1999. Bacterial interactions in early stages of marine cold water fish. Microbial

Ecology, 1: 1-26.

Hjelm, M., Bergh, O., Riaza, A., Nielsen, J., Melchiorsen, J., Jensen, S., Duncan, P., Ahrens, H., Birkbeck, H. and Gram, L. 2004. Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. System Appl. Microbial., 27: 360–371.

Ibrahim, F., Quwehand, A.C. and Salminen, S.J. 2004. Effect of temperature on *in vitro* adhesion of potential fish probiotics. Microbial Ecology in Health and Disease, 16: 222–227.

Irianto, A. and Austin, B. 2003. A short communication: use of dead probiotic cells to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. of Fish Diseases, 26: 59–62.

Irianto, A. and Austin, B. 2002. Probiotics in aquaculture: Review. Journal of Fish Diseases, 25: 633–642.

Irianto, A., Robertson, P.A.W. and Austin, B. 2000. The use of probiotics in aquaculture. Recent Research and Developments in Microbiology, 4: 557–567.

Irianto, A. and Austin, B. 2002. Use of probiotics to control furunculosis in rainbow trout, (*Oncorhynchus mykiss*, Walbaum). J. of Fish Diseases, 25: 333-342.

Jack, R.W., Tagg, J.R. and Ray, B. 1994. Bacteriocins of gram-positive bacteria. Microbiol. Rev., 59: 171–200.

Jones, P.J. and Hoffer, L.J. 2002. Clinical nutrition: 7. Functional foods: more than just nutrition. Canadian Medical Association Journal, 166: 1555–1559.

Jorborn, A. 1998. The role of the gasterointestinal microbiota in the prevention of bacterial infections in fish. PhD. thesis, Göteborg: Gothenburg university.

Kapetanovic, D., Kurtovic, B. and Teskeredzic, E. 2005. Differences in bacterial population in rainow trout (*Oncorhynchus mykiss*, Walbaum) fry after transfer from incubator to pools. Food Technol. Biotechnol., 43: 189–193.

Kim, D.H. and Austin, B. 2006. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. Fish & Shelfish Immunology, 21(5): 513–524.

Kozasa, M. 1986. Toyocerin (*Bacillus toyoi*) as growth promoter for animal feeding. Microbiology. Aliment. Nutr., 4: 121–135.

Nikoskelainen, S., Ouwehand, A.C., Bylund, G., Salminen, S. and Lilius, E.M. 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish & Shellfish Immnnology, 15: 443–452.

Panigrahi, A., Kiron, V., Kobayashi, T., Puangkaew, J., Satoh, S. and Sugita, H. 2004. Immune responses in rainbow trout, *Onchorhynchus mykiss*, induced by a potential probiotic, *Lactobacilus rhamnosus* JCM 1136. Veterinary Immunology and Immunopathology, 102: 379-388.

Rengpipat, S., Pukpratanporn, S., Piyatitivorakul, S., Menasaveta, P. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture, 191: 271–288.

Ringo, E. and Gatesoupe, F.J. 1998. Lactic acid bacteriain fish: a Review. Aquaculture, 160: 177–203

Ringo, E. and Storm, E. 1994. Microflora of free living fish and effect of diet and salinity on intestinal microflora. Aquaculture. Fish. Manage., 25: 623-629.

Ringo, E. and Birkbeck, T.H. 1999. Intestinal microflora of fish larvae and fry. Aquaculture Research, 30: 73–93.

Robertson, P.A.W., o'Dowd, C., Burrells, C., Williams, P. and Austin, B. 2000. Use of *Carnobacterium* sp. as probiotic for Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture, 185: 235-243.

Saarela, M., Mogensen, G., Fonden, R., Matto, J. and Mattila-Sadholm, T. 2000. Probiotics bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.

Sanders, M.E., Morelli, L. and Tompkins, T.A. 2003. Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. Comprehensive Reviews in Food Science and Food Safety, 2: 101-110.

Skjermo, J. and Vadstein, O. 1999. Tehniques for microbial control in the intensive rearing of marine larvae. Aquaculture, 177: 333-343.

Spanggaard, B., Huber, I., Nielsen, J., Nielsen, T., Appel, K.F. and Gram, L. 2000. The microflora of rainbow trout intestine: A Comparison of traditional and molecular identification. Aquaculture, 182: 1-15.

Sugita, H., Hirose, Y., Matsuo, N. and Deguchi, Y. 1998. Production of the antibacterial substance by *Bacillus* sp. strain NM12, an intestinal bacterium of Japanese coastal fish. Aquaculture, 165: 269-280.

Sugita, H., Miyajima, C. and Deguchi, H. 1991. The vitamine B_{12}-producing ability of intestinal microflora of freshwater fish. Aquaculture, 92: 267-276.

Sugita, H., Shibuya, K., Shimooka, H. and Deguchi, Y. 1996. Antibacterial abilities of intestinal bacteria in freshwater cultured fish. Aquaculture, 145: 195-203.

Taoka, Y., Maeda, H., Jo, J.Y., Jeon, M.J., Bai, S.C., Lee, W.J., Yuge, K. and Koshio, S. 2006. Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys oliraceus* to probiotics in a closed recirculating system. Fisheries Science, 72: 310-321.

Rosovitz, M.J., Voskuil, M.I., Chambliss, G.H. 1998. Bacillus. In: A. Balows and B.I. Duerden (Eds), Systematic Bacteriology. Arnold Press, London: 709-720

Verschuere, L., Rombout, G., Sorgeloos, P. and Verstraete, W. 2000. Probiotic Bacteria as Biological Control Agents in Aquaculture. Microbiology and Molecular Biology Reviews, 64: 655–671.

Vine, N.G., Leukes, W.D., Kaiser, H., Daya, S., Banter, J. and Hecht, T. 2004. Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. Journal of Fish Disease, 27: 319–326.

Vine, N.G., Leukes, W.D. and Kaiser, H. 2006. Probiotics in marine larviculture . FEMs Microbiol Rev., 30: 404-427.

Wache, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbe, L. and Quentel, C. 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow tout, *Onchorhynchus mykiss* fry. Aquaculture, 258: 470–478

Wang, Y-B., Xu, Z-R. and Xia, M.S. 2005 .The effectiveness of commercial probiotics in northern white shrimp Penaeus vannamei ponds. Fisheries Science, 71: 1036–1044.

Ziaei-Nejad, S., Habibi Rezaei, M., Azari Takami, G., Lovett, D., Mirvaghefi, A.R. and Shakouri, M. 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzymes activity, survival and growth in the Indian white shrimp Fenneropenaeus indicus. Aquaculture, 252: 516–524.