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The effects on growth and survival of probiotic *Bacillus* spp. fed to Persian sturgeon (*Acipencer persicus*) larvae

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Abstract. This study addressed if sturgeon larvae grew and survived better when fed a probiotic. *Bacillus* spp. were bioencapsulated in *Daphnia magna* (Straus 1820) and fed to *Acipencer persicus* (Borodin 1897) larvae. *Bacillus* bacteria (three species in a commercial preparation, Protexin Aquatic) were bioencapsulated within *Daphnia* at three concentrations by holding the *Daphnia* in suspensions of 1×10^7, 2×10^7or 3×10^7 bacteria per milliliter for 10 hours. The sturgeon larvae were fed one of the three probiotic treatments at a level of 30 percent body weight 5 times a day. The growth and survival of larvae fed the protbiotic enriched Daphnia were compared to those larvae fed a control treatment of unbioencapsulated *Daphnia magna*. The results showed that larvae fed the probiotic *Bacillus* spp. had increased final body weight and specific growth rate in comparison to control treatment. The probiotic bacillus also had significant positive effects on daily growth ratio, daily growth coefficient, average weight gain and survival in comparison to those fed the control treatment. The food conversation ratio was the one factor that decreased significantly in comparison with the control treatment. The amount of probiotic used did not improve performance, so larviculture can be increased merely by the addition of of low level of probiotics.

Key Words: Probiotic, bioencapsulation, *Daphnia magna*, growth, *Acipencer persicus*.

چکیده: در این مطالعه مشخص شد که اگر لارو خاویاری از پروبیوتیک تغذیه کند دارای رشد و بازماندگی بیشتری است. گونه های باسیلوس در دافنی ماگنا (Straus 1820) غنی سازی شدند و به لارو قره برون (Borodin 1897) خورانده شدند. باکتری های باسیلوسی (سه گونه در یک بسته تجاری پروتوکسین) در دافنی در سه غلظت 1×10^7، 2×10^7 یا 3×10^7 باکتری به ازائ هر میلی لیتر در 10 ساعت غنی سازی شدند. لاروهای قره برون به نسبت 30 درصد وزن بدن در پنج نوبت در روز تغذیه شدند. رشد و بازماندگی لاروهای تغذیه شده با دافنی غنی سازی شده با لارو های تغذیه شده با گروه کنترل مقایسه شدند. نتایج نشان دادند که در لاروهای تغذیه شده با باسیلوس های پروبیوتیکی وزن نهایی و نرخ رشد ویژه در مقایسه با گروه کنترل افزایش یافت. همچنین باسیلوس های پروبیوتیکی تاثیرات مثبت معنی داری روی نرخ رشد روزانه، ضریب رشد روزانه، میانگین وزن بدست امده و بازماندگی در مقایسه با گروه کنترل داشتند. ضریب تبدیل غذایی در تیمارهای آزمایشی در مقایسه با گروه کنترل به طور معنی داری کاهش یافت. مقدار زیاد پروبیوتیک های استفاده شده عملکردلاروها را افزایش نداد، اگرچه عملکرد لاروها می تواند با اضافه کردن مقدار کم پروبیوتیک هم افزایش بیابد.

Introduction. Probiotics are a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal balance and health of the host (Fuller 1986). Most studies with probiotics conducted to date in fish have been undertaken with microbial strains isolated and selected from aquatic environments. There are a wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*), gram positive (*Bacillus*, *Lactococcus*, *Micrococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Streptcoccus*, *Weisslla*) and gram negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* and *Vibrio*) that have been evaluated as a probiotic in aquaculture (Gastesoupe 1999). The appropriate use of probiotics in the aquaculture industry were shown to improve intestinal microbial balance, and also to improve feed absorption, thus leading to increased growth rate (Fuller 1989; Rengpipat et al 1998) and also reduced feed conversion ratio (FCR) during the cultural period (Wang et al 2005).

Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathenogenic 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 (Verschuere et al 2000). Because *Bacillus* bacteria secrete many exoenzymes (Moriarty 1998), these bacteria have been used widely as putative probiotics.

The present study examined the effects of probiotic *Bacillus* spp. on growth and survival in persian sturgeon (*Acipencer persicus* Borodin 1897) larvae, when the *Bacillus* spp. were bioencapsulated within *Daphnia magna* (Straus 1820).

Material and Method

Preparing of probiotic Bacillus. The probiotic *Bacillus* was prepared from the commerical product Protexin aquatic (Iran-Nikotak), which is a blend of three *Bacillus* species. The blend of probiotic *Bacillii* (*B. licheniformis, B. subtilis* and *B. circulans*) from suspension of spores with special media were provided. Three concentrations of bacterial suspensions, 1×10^7, 2×10^7 and 3×10^7 bacteries per milliliter (CFU mL^{-1}) were provided by Protexin Co and the colony forming unit (CFU) of probiotic *Bacillii* were tested by microbial culture in Tryptic Soy Agar (TSA) (Rengpipat et al 1998).

***Daphnia magna* removal and bioencapsulation**. *Daphnia magna* were obtained from intensive production ground ponds of the center of sturgeon culture of Marjani (Iran). The *Daphnia magna* at a density of 5 g live *Daphnia* $litter^{-1}$ was held in a broth suspension with *Bacillus circulans*, *Bacillus subtilis* and *Bacillus licheniformis* at densities of 1×10^7, 2×10^7 and 3×10^7 bacteria per milliliter for 10 hours.

Experimental design. This experiment was conducted in a completely randomized design with four treatments (three probiotic levels and a control), and three replicates per treatment for a total of twelve fiberglass tanks (each with a capacity of 40 liters). Larvae of Persian sturgeon (initial weight: 74.9±0.89 mg) were obtained from the center of sturgeon culture of Marjani (Iran). The density of fish larvae in per tank were 71 fish. Persian sturgeon larvae in control and experimental treatments were fed 30 percent of their body weight for 5 times a day (2.00, 7.00, 12.00, 17.00 and 22.00). The control treatment was fed unbioencapsulated *D.magna*. Water quality parameters of input water to rearing system were monitored each week throughout the experimental. The water temperature was 19.46±1.23°C, pH was 7.85±0.26 and water oxygen level was maintained above 7.65 ± 0.55 mg L^{-1} during the experiment an electrical air pump (by a single filtration unit).

Sample collection. The fish were weighed individually at the beginning and at the end of the experiment. Before distributing fish to the experimental tanks (in the beginning of exogenous feeding), 30 fish were sampled from the holding tank for biometry. In the termination of experiment, 50 larvae from each tank were sampled and the final weight and length of body were measured.

Calculation and statistical analysis. Growth and feeding parameters of fish were calculated based on the data of biometry of Persian sturgeon larvae, and included:

Food Conversion Ratio (FCR) = Total feed consumed (g)/ ($n_{initial}$-n_{final}) (De Silva & Anderson 1995).
Specific growth rate (SGR (%Body weight day^{-1})) = $[(\text{Ln } BWt_1 – \text{Ln } BWt_0) / t_1 - t_0] \times 100$
Specific growth rate (SGR (%Body length day^{-1})) = $[(\text{Ln } BLt_1 – \text{Ln } BLt_0) / t_1 - t_0] \times 100$
Average weight gain (AWG %)= $[(BWt_1 - BWt_0)/BWt_0)]\times100$ (De Silva & Anderson 1995).
Daily growth coefficient (DGC)= $100x(BWt_1^{1/3} – BWt_0^{1/3})$ (Cho 1992).
Condition Factor (CF) = $BWt_1\times100/L^3$ (Ai et al 2006).
Daily growth rate (DGR) = $\{100\times(BWt_1 - BWt_0)/((t_1–t_0)x BWt_0)\}$ (De Silva & Anderson 1995).
Survival (%) = n_{final} / $n_{initial}$ *100 (Ai et al 2006).

For all equations, BWt_0 and BWt_1 are initial and final body weight of fish larvae and $t_1 - t_0$ is duration of experiment (De Silva and Anderson 1995). Number of fish is indicated as initial ($n_{initial}$) and final (n_{final}). In calculating the specific growth rate, $LnBWt_0$ and $LnBWt_1$ are the natural (neperian) logarithm of initial and final body weight, where $LnBLt_0$ and $LnBLt_1$ are neperian logarithms of initial and final body length of fish larvae, and $t_1 - t_0$ is duration of experiment (De Silva & Anderson 1995). Body weight increase was expressed as: BWI (mg) = $BWt_1 - BWt_0$ (Tacon 1990) where BWt_0 and BWt_1 are initial and final body weight of fish larvae.

One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

Results. The results clearly showed that the *Bacillus* enrichment had beneficial effects on the growth parameters in *Acipenser persicus* larvae. The feeding and growth parameters of Persian sturgeon larvae are presented in Table 1. All the probiotic treatments resulted in growth performance and survival better than that of the controls ($p < 0.05$). The three different treatments of probiotic were not statistically different for any of the growth parameters. However, among the three different concentrations of probiotic *Bacillus* fed bioencapsulated in *Daphnia magna to* Persian sturgeon larvae, the greatest effect appeared to be obtained in treatments 2 and 3 (bioencapsulated *Daphnia* with 2×10^7 and 3×10^7 CFU/ mL). This is particularly true for average of weight gain, where the highest was obtained in the experimental treatment of T2 and T3. Of note is that food conversion ratio (FCR) in the experimental treatments was nearly half that of the control treatment ($p<0.05$). Bacillus enrichment had significant positive effect on survival by 15 to 20%.

Table 1

Growth parameters of Persian sturgeon (*Acipencer persicus*) larvae in experimental treatments (trial 1-3) and control

Growth Indices	Control Unbioencapsulated *Daphnia magna*	T1 Bioencapsulated *Daphnia magna* with 1×10^7 CFU/ mL	T2 Bioencapsulated *Daphnia magna* with 2×10^7 CFU/ mL	T3 Bioencapsulated *Daphnia magna* with 3×10^7 CFU/ mL
Initial weight (mg)	74.9±9.71	74.9±9.71	74.9±9.71	74.9±9.71
Final body weight (mg)	388.01±55.24^b	617.16±72.44^a	640.10±86.59^a	640.81±87.90^a
Body weight increased (mg)	313.11±54.25^b	542.26±72.44^a	565.20±86.59^a	565.91±87.90^a
Average weight gain (%)	418.04±55.95^b	723.958±30.23^a	754.61±49.12^a	755.55±50.86^a
Specific growth rate for weight (% BW day^{-1})	5.53±1.65^b	7.36±1.17^a	7.50±1.11^a	7.49±1.13^a
Specific growth rate for length (% BL day^{-1})	1.66±0.23^b	2.37±0.41^a	2.51±0.54^a	2.44±0.42^a
Daily growth coefficient (%)	2.92±0.39^b	4.20±0.86^a	4.30±0.85^a	4.30±0.8^a
Daily growth Ratio	14.92±2.35^b	25.85±2.22^a	26.95±2.89^a	26.98±2.95^a
condition Factor	559.25±89.33^a	537.60±41.63^b	522.81±67.88^b	530.55±74.85^b
Feed Conversion Ratio	8.48±0.33^a	4.92±0.15^b	4.73±0.50^b	4.71±0.15^b
Survival	79.99±5.71^b	94.28±4.28^a	96.18±2.18^a	98.09±2.18^a

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Discussion. In this study, *Daphnia magna* were used as a vector to carry the probiotic *Bacillus* to the digestive tract of Persian sturgeon larvae. The probiotics in this experiment promoted the feeding and growth parameters in Persian sturgeon larvae in experimental treatments in comparison to control treatment.

Effects of commercial probiotic on aquaculture has been investigated by researchers, and some of this research has not shown any positive effects on growth parameters or survival rate or any promising result on the cultural condition. For instance, Shariff et al (2001) found that treatment of *Penaeus monodon* with a commercial *Bacillus* probiotic did not significantly increase survival. These results disagree with our findings, although fish and crustaceans may respond differently to probiotics.

Results of all the probiotic treatments showed better growth performance and some of feeding parameters than the control. The beneficial effect of probiotc *Bacillus* pp.

on the feeding efficiency of *Asipencer persicus* larvae was completely observed. The results indicated that the probiotic bacillus had significantly effects on the growth and feeding parameters in experimental treatments. The better body weight and SGR for weight and length were obtained in experimental treatments. Similar finding were observed by Gatesoupe (1991) in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus, 1758), where Swain et al (1996) in Indian carps that improved the growth factors and feeding performance and Ghosh et al (2003) on the Rohu.

Bagheri et al (2008) 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. This finding agrees with our results. Ghosh et al (2002) indicated that the *B. circulans*, *B. subtilis* and *Bacillus pamilus*, isolated from the gut of Rohu, have extracellular protease, amylase, and cellulose and play an important role in the nutrition of Rohu fingerlings. The photosynthetic bacteria and *Bacillus sp*. (isolated from the pond of common carp) was used in diet of common carp (*Cyprinus carpio* Linnaeus, 1758) by Yanbo & Zirong (2006). The results indicated that this probiotics increased growth parameters and digestive enzyme activities. The results of these studies showed that the blend,of bacterial probiotics can increase the growth and feeding efficiency in fish of experimental treatments in comparison of control. Also the results were indicted that using different levels of probiotic *Bacillus* in bacterial suspension of bioencapsulated *Daphnia* had different (but not significant) results on growth and feeding parameters of Persian sturgeon larvae. *Daphnia magna* are likely to be limited in their potential to carry of bacteria from a bioencapsulation broth to the digestive tract of fish larvae.

Conclusions. This experiment demonstrated that the probiotic *Bacillus* have the ability to improve the growth parameters in *Acipenser persicus* larvae. Different concentrations of probiotic bacilluse did not have statistically different effects on the growth and feeding parameters in Persian sturgeon larvae. Overall, probiotics can be useful in the improving the performance of larviculture of this species.

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