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Probiotic Performance on Fish Fry during Packing, Transportation Stress and Post-transportation Condition

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Abstract: An experiment was conducted to determine whether the application of probiotic after transportation provides the same benefit of probiotic application prior to/during transportation in improving fry survival and growth. Fry of the Indian major carp *Catla catla* were stocked into 2 tanks (capacity: 2000 L) for conditioning. During this time one tank was treated with probiotic at the rate of 10 ppm for 2 h. The other tank was not treated with probiotic. The fry from both treatment and control tanks were packed in plastic bags at a packing density of 400 fry L^{-1}. The fry that previously received probiotic treatment were packed in water containing probiotic whereas the fry from the control tank was packed in water without probiotic. The bags were transported to the laboratory and unpacked after 13 h. Treatment with probiotic resulted in higher survival (98.4%) whereas fry not treated with probiotic had a mean survival of 92.5%. Post-transportation, the fry in each bag were stocked in individual, shallow, plastic tanks at a density of 25 fry L^{-1} and reared for five days. The fry that were administered probiotic during transportation were either treated with probiotic or not treated during this period. Similarly, the fry that were not administered probiotic during transportation, were either treated with probiotic or not treated. Wherever applicable, probiotic was added at the rate of 10 ppm per day after water exchange, but before first feeding. They were fed *ad libitum* three times per day. Fry that were treated with probiotic throughout the trial gave the highest survival (95.2%) and growth (mean final weight = 1060 mg).

Key words: Probiotics, bacteria, yeast, feed, survival

INTRODUCTION

Packing and transportation of fish fry in hatcheries cause stress which results in mortalities and reduced performance (Carneiro and Urbinati, 2002; Gomes *et al*., 2003; Taoka *et al*., 2006). Transportation stress is one of the primary contributing factors of fish disease and mortality in aquaculture (Rollo *et al*., 2006). Many factors affect the survival of fish during transportation. Water exchange to provide oxygen for respiration and removal of toxic metabolites such as carbon dioxide and

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ammonia is limited during transportation. Fish are often starved (also called conditioned) before packing to lower its metabolism (Gomes *et al*., 2002). Temperature of the water is also reduced to lower the metabolism. The fish are concentrated to permit more efficient transportation. Handling, crowding and poor water quality imposes severe stress on the fragile fry and fingerlings of fish (Iversen *et al*., 1998).

The commercial probiotic (named Efinol® L; manufactured by Bentoli, Inc., USA) is a product for lowering stress response prior to and during transport of live fish fry and fingerlings. This probiotic is a combination of microbial cultures and selected nutrients for use in shrimp, fish and reptilian hatcheries. This combination has been scientifically shown to counteract the stressful conditions of hatcheries and improve health and survival of aquatic animal larvae. Investigations on the application of this product on freshwater fish in tropical conditions are limited. A series of trials were conducted to test the effect of probiotic on the survival of freshwater fish fry and fingerlings prior to, during and after transportation. The Indian major carp, *Catla catla*, which is known to be sensitive to transportation stress, was used as a model species in the trials. In a previous trial, we found that probiotic application prior to packing stress improved the survival and post-transportation growth of the carp fry. We wanted to determine whether post-transportation treatment with probiotic would further improve fry performance. We also wanted to determine whether post-transportation treatment alone would provide improvement in fry performance when compared to treatment prior to and during transportation.

MATERIALS AND METHODS

Catla catla fry [initial Total Length (TL) = 1.97 cm and initial total weight (BW) = 800 mg] were collected from Subam Aquafarm, Kalidaikurichi, India and stocked into 2 tanks (capacity: 2000 L) for conditioning. During this time one tank was treated with probiotic at the rate of 10 ppm for 2 h. The other tank was not treated with probiotic. The fry from both treatment and control tanks were packed in plastic bags with at a density of 400 fry L^{-1}. The fry that previously received probiotic treatment were packed in water containing probiotic whereas the fry from the control tank was packed in water without probiotic. Six replicates (bags) were maintained for each treatment. The individuals were packed in the late evening (05:30 pm) and transported to the Center for Aquaculture Research and Extension (CARE), Palayamkottai, India, where the trial was conducted during August 2006. After arriving at CARE, the bags were left unpacked for 13 h. They were unpacked next day morning at 07:00 am. Survival was recorded immediately after unpacking. Fry from each bag was transferred to individual, shallow, plastic container (capacity: 60 L) at 25 fry L^{-1}. A post-stocking growth trial was conducted in the following manner (Fig. 1):

Treatment A

Fry that were treated with probiotic prior to and during transportation were treated with probiotic post-transportation (probiotic/probiotic).

Treatment B

Fry that were treated with probiotic prior to and during transportation were not treated with probiotic post-transportation (probiotic/no probiotic).

Treatment C

Fry that were not treated with probiotic prior to and during transportation were treated with probiotic post-transportation (no probiotic/probiotic).

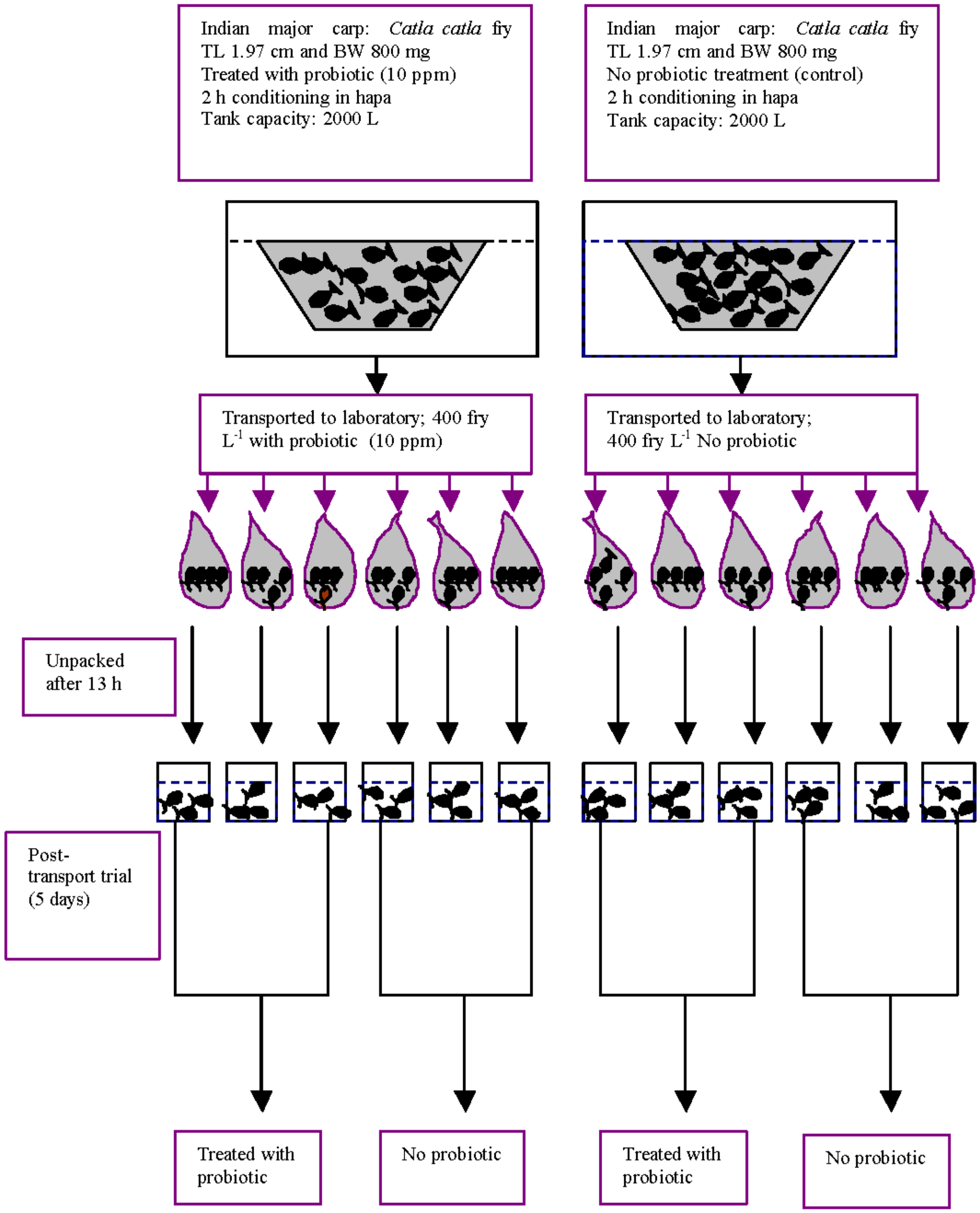

Fig. 1: Schematic diagram showing trial methodology

Treatment D

Fry that were not treated with probiotic prior to and during packing were not treated with probiotic post-transportation (no probiotic/no probiotic).

Probiotic was added at the rate of 10 ppm per day after water exchange, but before first feeding. The fry were fed *ad libitum* three times (08:30, 13:30 and 18:30 h) per day. The unfed feeds were siphoned out before feeding. Growth and survival were monitored for five days post-transportation.

RESULTS AND DISCUSSION

In the present study, treatment with probiotic prior to and during transportation resulted in higher fry survival immediately after transportation. Fry in water containing probiotic had a mean survival of 98.4% whereas fry not treated with probiotic had a mean survival of 92.5% (Table 1). Continuing probiotic treatment after transportation improved fry survival (Table 2) and fish size. Survival of the fry receiving probiotic was the highest survival (95.2%) and greatest final fry weight (1060 mg) five days post-transportation. Fry that were not treated with probiotic at any time during the trial gave the poor survival (89.5%) and lowest final weight (820 mg). Survival and final mean weight of fry that were treated with probiotic only prior to and during transportation were 91.3% and 970 mg, respectively and were marginally higher than those fry that were treated with probiotic only after transportation (91.2% and 930 mg, respectively). The growth and survival data of probiotic application before, during and after transportation were statistically significant with no application of probiotics.

Transportation stress is one of the primary contributing factors of fish disease and mortality in aquaculture. Under natural conditions fish often experience brief periods of stress, bringing about a temporary disturbance of homeostasis (Van-Weerd and Komen, 1998). Probiotics are inoculated into the rearing water to improve culture conditions or incorporated in the food of fish through diet (Nogami *et al*., 1997; Moriarty, 1998; Corre *et al*., 2001).

Probiotics composed of microbial cultures of yeast and bacteria widely used to improve the health of humans and livestock (Irianto and Austin, 2002). In black tiger shrimp, scallop, flounder, Atlantic salmon, rainbow trout and turbot, the growth and survival plus water and sediment quality of the farm environment were improved and the microbial flora in fish intestine, rearing water and feces were stabilized by addition of probiotics (Gatescope, 1994; Riquelme *et al*., 1997; Byun, 1997; Rengpipat *et al*., 1998; Robertson *et al*., 2000). The influence of microbial flora from the rearing water on the gastrointestinal flora of the cultured animal is widely recognized (Sugita *et al*., 1981).

Rengpipat *et al*. (1998) reported that probiotics added to shrimp diets did not affect water quality. However, when probiotics was added to the rearing water, the water quality slightly improved as compared to the case without probiotics (Corre *et al*., 2001; Chen and Chen, 2001). The inoculation of probiotics to the water during the transport in the present experiment remarkably influences water quality in terms of survival and growth.

Table 1: Percentage survival of *Catla catla* treated with probiotic after 13 h of packing/transportation stress

Bag No.	Treated with probiotic	Not treated with probiotic
1	98.00^a	93.25^b
2	98.25^a	92.50^b
3	99.00^a	91.75^b
4	99.00^a	92.50^b
5	98.00^a	92.75^b
6	98.25^a	92.50^b
Average	98.41	92.54
SD	0.46	0.48

Different alphabet between the row statistically significant at $p<0.05$ by students, t-test

Table 2: Cumulative survival (%) of *Catla catla* fry during the post-transportation trial

No. of days	Probiotic/ Probiotic	Probiotic/ No Probiotic	No Probiotic/ Probiotic	No Probiotic/ No probiotic
1	96.44^a	95.94^a	94.23^b	95.21^a
2	96.35^a	94.90^a	93.87^c	94.67^a
3	95.84^a	92.95^b	92.88^b	92.06^b
4	95.67^a	91.59^c	92.52^b	90.98^c
5	95.25^a	91.25^b	91.17^b	89.54^c

Different alphabet between the row statistically significant at $p<0.05$ by students, t-test

The introduction of probiotics into the water before and during transport appeared to enhance not only survival but also growth of *C. catla* fry. The similar result was achieved on Japanese flounder by Taoka *et al*. (2006). Post transportation growth trials also showed that the introduction of probiotics to the rearing water accelerated the growth of *C. catla* and improved the survival significantly. The survival rate in the probiotic group was significantly higher than that in the control group. So the transportation stress tolerance of fish is enhanced by probiotics. The present study showed that probiotic treatment enhances not only the transport stress tolerance in cultured fish but also increased survival, growth and resistance to the pathogen by probiotics has been widely reported.

The probiotics application, as proved here, enhanced the resistance of the organisms to chemical-physical changes and to pathogen infections, as previously observed in other species (Westerdahl *et al*., 1991; Havenaar *et al*., 1992; Salminen *et al*., 1998), suggesting that probiotics might be a good alternative to the use of antibiotics in aquaculture. The results obtained in this study represent the first step towards the development of an environmentally friendly aquaculture. Moreover, since the products of aquaculture are devolved to human consumption, the use of probiotics as a live microbial feed supplement may represent a guarantee of a safer product for human health. The relevance of this study is that different from most of the work so far carried out on the use of probiotics. The treatment with probiotic improves the performance of fish fry after packing and transportation stress, but the magnitude of performance improvement depends on the length and timing of application

CONCLUSION

The trial clearly demonstrated the benefit of using probiotic in fish hatcheries. probiotic application prior to and during transportation increases fry survival immediately and for at least five days post-transportation. Extending probiotic application beyond transportation extended the effect of probiotic on fry survival. For this reason, probiotic use is recommended prior to, during and after the occurrence of stress. Probiotic is equally effective when used either ahead of transportation or after transportation. However, use ahead of transportation is recommended for reducing mortalities in transport. For the best results, probiotic should be used ahead of, during and after transport. Probiotics treatment also improves the growth of fish fry. This effect is probably because the treated animals are able to recover much faster from the stress than their untreated counterparts.

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REFERENCES

Byun, J.W., 1997. Probiotic effect of *Lactobacillus* sp. Ds-12 in flounder. J. Gen. Applied Microbiol., 43: 305-308.

Carneiro, P.C.F. and E.C. Urbinati, 2002. Transport stress in matrinxã, *Brycon cephalus* (Teleostei: Characidae), at different densities. Aqua. Int., 10: 221-229.

Chen, C.C. and S.N. Chen, 2001. Water quality management with *Bacillus* sp. in the high-density culture of red-parrot fish *Cichlasona citrinellum* x *C. sunspilum*. N. Am. J. Aqua., 63: 66-73.

Corre, V. Jr., R.J. Janeo and A.M.A. Caipang, 2001. Use of probiotics, reservoir with green water in shrimp farming in the Philippines. Proceed. Jpn. Soc. Promotion Sci. (JSPS) 30: 67-72.

Gatescope, F.J., 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic *Vibrio*. Aqua. Living Res., 7: 277-282.

Gomes, L.C., R.Roubach and C.A.R.M. Araujo-Lima, 2002. Transportation of tambaqui juveniles (*Colossoma macropomum*) in Amazon: Main problems. World Aqua., 33: 51-53.

Gomes, L.C., C.R.M. Araujo-Limrao, D.A. Oubach, C. Gomeansd and N.P. Lope, 2003. Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. J. World Aqua. Soc., 34: 76-84.

Havenaar, R., B. Ten Brink and J.H.J. Huisin Veld, 1992. Selection of Strains for Probiotics Use. In: The Scientific Basis Fuller, R. (Ed.), Probiotics. Chapman and Hall, London, pp: 209-224.

Irianto, A. and B. Austin, 2002. Probiotics in aquaculture. J. Fish Dis., 25: 633-642.

Iversen, M., B. Finstad and K. Nilssen, 1998. Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture, 168: 387-394.

Moriarty, D.J.W., 1998. Control of luminous *Vibrio* species in penaeid aquaculture pond. Aquaculture, 164: 351-358.

Nogami, K., K. Hamasaki, M. Maeda and K. Hirayama, 1997. Biocontrol method in aquaculture for rearing the swimming crab larvae *Portunus trituberculatus*. Hydrobiologia, 358: 291-295.

Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul and P. Menasveta, 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture, 167: 301-313.

Riquelme, C., R. Araya, V. Nelson, A. Rojas, M. Guaita and M. Candia, 1997. Potential probiotic strains in the culture of the Chilean scallop *Argopecten purratus* (Lamarck, 1819). Aquaculture, 154: 17-26.

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

Rollo, A., R. Sulpizio, M. Nardi, S. Silvi, C. Orpianesi, M. Caggiano, A. Cresci and O. Carnevali, 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiol. Biochem., 32: 167-177.

Salminen, S., C. Bouley, M.C. Boutron-Ruault, J.H. Cummings, A. Franck, G.R. Gibson, E. Isolauri, M.C. Moreau, M. Roberfroid and I. Rowland, 1998. Functional food science and gastrointestinal physiology function. J. Nut., 80: S147-S171.

Sugita, H., H. Tanaami, T. Kobashi and Y. Geguchi, 1981. Bacterial flora of coastal bivalves. Nippon Suisan Gakkaishi, 47: 655-661.

Taoka, Y., H. Maeda, J.Y. Jo, M. J. Jeon, S.C. Bai, W.J. Lee, K. Yuge and D.S. Oshio, 2006. Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* toprobiotics in a closed recirculating system. Fish. Sci., 72: 310-321.

Van-Weerd, J.H. and J. Komen, 1998. The effects of chronic stress on growth in fish: A critical appraisal. Comp. Biochem. Physiol. Part A, 120: 107-112.

Westerdahl, A., J.C. Olsson, S. Kjelleberg, P.L. Conway, 1991. Isolation and characterization of turbot (*Scophtalmus maximus*) associated bacteria with inhibitory effects against *Vibrio anguillarum*. Applied Environ. Microbiol., 57: 2223-2228.