Review paper

# USE OF PROBIOTICS AS GROWTH PROMOTERS AND IMMUNOSTIMULATORS IN FINGERLINGS OF CYPRINID FISH SPECIES

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**Abstract**

Intensive aquaculture production has required the development of an individual's resistance to disease rather than depending upon antibiotics or chemotherapeutics. The role of gastrointestinal microflora in disease resistance has been established in many fish species, which has led to the concept of manipulating gastrointestinal microflora for better health management. A number of studies has been conducted in different fish species with various useful microorganisms called 'probiotics' to amplify gastrointestinal microflora to fight against various infectious diseases. Probiotics are beneficial microorganisms which protect the host from diseases. Probiotic protection can be achieved by various mechanisms. Most probiotics used in aquaculture belong to the lactic acid bacteria, the genus *Bacillus*, the photosynthetic bacteria, the yeast, notwithstanding other genera and species have also been used. The immunostimulatory effect of probiotics has been established in many fish species, but their direct involvement in the immune response is not well established. It has also been proven that the application of probiotics in aquaculture has beneficial effects on growth of fish as well as on the environment. At present, data about the efficacy of probiotics in commercial aquaculture of Serbia is still lacking. This review discusses mainly the studies and applications about effects, problems and perspectives of probiotics used in fingerlings of cyprinid fish species, and highlights immunostimulatory effects and growth promotion effects of commercial probiotic products. In the present paper the results that show positive influence of probiotics in cyprinides nutrition on production performance and immune system are summarized. Special accent is given to criteria for proper selection of probiotics in cyprinides production.

**Key words:** *cyprinids, growth promoters, immune system, probiotics*

## Introduction

In the several past years, the usage of some antibiotics has been forbidden by several countries due to serious environmental hazards and various adverse effects in many fish species (Gatesoupe, 2007). Furthermore, resistence of pathogen could increase which often leads to spreading of disease, especially under some stressful culture conditions or if antibiotics are used in lower doses for economical reasons (Suzer et al., 2008). Functional additives, like probiotics, represent a new concept on aquaculture. Probiotics have been

increasingly used in aquaculture after their first admission (Kozasa, 1986). Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotics as live micro-organisms that when administered in appropriate amount confer a health benefit on the host (FAO/WHO, 2001). Probiotics supplemented in fish feed resulted in growth performances and feed utilization better than that of the control basal feed, suggesting that the addition of probiotics reduced the culture cost of cyprinid fish species (Jovanović et al., 2011; Miščević et al., 2011 and 2012). In addition, the application of probiotics in aquaculture as the environment friendly treatments was also increasing rapidly (Gatesoupe, 1999). The immunostimulatory effect of probiotics has been also observed in many studies on different fish species (Salinas et al., 2008). Probiotics should also be considered as potentially useful for the control of fish diseases.

At present, data about the efficacy of probiotics in commercial aquaculture of Serbia are still lacking. This review discusses mainly the studies about applications, effects, problems and perspectives of probiotics used in fingerlings of cyprinid fish species, and highlights immunostimulatory effects and growth promotion effects of commercial probiotic products. In the present paper the results that show positive influence of probiotics in cyprinides nutrition on production performance and immunological system are summarized. Special accent is given to criteria for proper selection of probiotics in cyprinides production.

## Probiotics

Probiotics represent components of a micro-organism that is beneficial to the health of the host, so they include certain bacteria strains and yeasts that are not adverse on continued use for a longer period of time (Irianto and Austin, 2002). The mode of action is that good and useful microbes multiply in order to compete with the harmful ones, thus suppressing their growth (Sahu et al., 2008). Wang et al. (2008) suggested that the definition of a probiotic in aquaculture should include the addition of live naturally occurring bacteria to ponds in which fish live. Most probiotics used in aquaculture belong to the genus *Bacillus*, *Lactobacillus* sp., *Bifidobacterium* sp., *Vibrio* sp., *Saccharomyces* sp., *Enterococcus* sp., the photosynthetic bacteria, the yeast, (Kumar et al., 2006) notwithstanding other genera and species that have also been used. Fuller (1987) noted that a probiotic should provide actual benefit to the host, be capable to survive in the digestive tract, be suitable for commercialization and be stable and viable for long-term storage conditions.

## Probiotics as growth promoters in fingerlings of cyprinid fish species

Growth process in fish depends on numerous factors (Azaza et al., 2008; Deane and Woo, 2009). One of the most important factors on growth and health of fish is feed supply (Lall and Lewis-McCrea, 2007). According to DeVrese and Marteau (2007), mechanism and function of probiotics effect depend principally on the interactions between probiotic species and microbiota of the host or with immuno competent cell of the intestinal mucous. The growth of fish might have occurred by probiotics interaction that increased minerals absorption (Tewe et al., 1999). Jovanović et al. (2011), Miščević et al. (2011 and 2012) observed that the adding commercial probiotics in the diets for common carp fingerlings improve weight of fish. Results obtained by Faramarzi et al. (2011) indicate that the fingerlings of common carp fed with diets with probiotics supplemented exhibited greater growth than those fed with the control diet. Of the four probiotics treatments in the mentioned trial, the 40% protein diet supplemented with yeast showed the best growth

performance and feed efficiency, suggesting that yeast is an appropriate growth stimulating additive in common carp cultivation. Sahandi et al. (2012) also reported the similar results that demonstrate the effect of *Bacillus* spp on length of silver carp and increased fish growth. Effect of probiotics for common carp (*Cyprinus carpio* L.) and some other warm-water fish species based on growth performance and feed utilization (feed conversion ratio, FCR) were investigated also by Wang and Xu, 2006; Wang et al., 2008; Lara-Flores et al., 2010). The above mentioned authors established that the use of probiotics improve growth performance and show the growth promoting effect. All the probiotics supplemented diets resulted in growth performance and feed utilization better than that of the control diets, suggesting that the addition of probiotics reduced the culture cost of common carp (*Cyprinus carpio*). The use of probiotics was suggested as a tool for improving the growth performance and increasing feed digestibility (Sahandi et al., 2012; Ljubojević et al., 2013). Increase of the weight of fish under the probiotics consumption is not completely understood, but according to Lilly and Stillwell (1965) probiotics improve the gastrointestinal microbial populations and then increase enzyme secretion (Suzer et al., 2008) and consequently increase utilization of nutrients and protein anabolism as the length and weight gain occurs in fish.

**Probiotics as immunostimulators in fingerlings of cyprinid fish species**

Probiotics are used as dietary supplementations in aquaculture and their role in intestinal microbial balance, growth, nutrition, health status and resistance against infectious agents are already established (Gatesoupe, 1999; Ljubojević et al., 2013). Although, some reports are available on the activation of the immune response by dietary supplementation with probiotics in fish (Aly et al., 2008) their direct involvement in the immune response is not well established (Geijtenbeek and Gringhuis, 2009). Nayak et al. (2007) determined the effect of dietary supplementation with the probiotics bacterium, *Bacillus subtilis*, vitamin C in the form of ascorbyl polyphosphate and their combination on the immune response and disease resistance of Indian major carps. The non-specific immune system can be stimulated by probiotics (Balcázar et al., 2006). In the study conducted by Kumar et al. (2006), an increased growth rate was observed in *L. rohita* (Indian carp) fed diet containing *B. subtilis* compared with control. Furthermore, the survival rate after challenge with *A. hydrophila* was significantly higher in the treatment group compared with the control. Administration of probiotics enhances the survival of carp infected with *A. hydrophila* (Selvaraj et al., 2005). The high rates of establishment of bacterium in the gastro-intestinal tract of fish treated with *B. subtilis* have suppressed the *A. hydrophila* infection, which ultimately resulted in the higher survival (Kumar et al., 2006).

**Beneficial effects of probiotics on the environment**

Probiotics could help in improvement of the water quality in fish ponds (Panigrahi and Azad, 2007). This is due to the capability of the probiotic bacteria to participate in the turnover of organic nutrients in the ponds, to reduce ammonia, nitrite and total nitrogen levels in water and thus improve the chemical quality of water (Zhang et al., 2013).

**Proper selection of probiotics in cyprinides production**

For proper selection of probiotics it is essential to understand the mechanisms of their action and to define selection criteria for potential probiotics (Klaenhammer and Kullen,

1999). According to Sahu et al. (2008) methods to select probiotic bacteria for use in the aquaculture should include the following steps: collection of background information, acquisition of putative probiotics, screening of putative probiotics, evaluation of pathogenicity and survival test, *in vivo* evaluation, and evaluation of their effects in rearing conditions.

## Future perspectives

Generally, the study of probiotics in fish production of Serbia is still in its early stage, and there have been no commercial probiotics products produced in Serbia so far. In the future, probiotics will gain more popularity in aquaculture of the Republic of Serbia, and the application areas will be expanded. Thus, quality control of probiotics in aquaculture of the Republic of Serbia will become an important issue. The need for the development of adequate technology for the evaluation of the efficiency of microbial agents as probiotics in aquaculture will be also increased.

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