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USE OF PROBIOTIC BACTERIA IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AQUACULTURE: SHORT COMMUNICATION

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ABSTRACT

In recent years, there has been a growing interest in controlling disease-related problems through alternative methods, since the use of chemotherapeutic agents may lead to occurrence of resistant bacteria. This short communication summarizes the current understanding of probiotic use in aquaculture of rainbow trout to prevent pathogenic bacteria, including the definition and mechanism of probiotics action, and describes their application, prospects and difficulties associated with their use in aquaculture. Our contribution to the use of probiotic bacteria in aquaculture represents isolation of lactic acid bacteria (LAB) from the intestinal content of rainbow trout (*Oncorhynchus mykiss*), subsequently potentially used as probiotics in order to improve health status of fish during fish farming. An effective probiotic must comply with criteria which determine its effect. Selection criteria are used to obtain suitable probiotic candidates for aquaculture including antimicrobial susceptibility test, determination of *in vitro* and *in vivo* survival conditions in the gastrointestinal tract of rainbow trout, and tolerance to different pH values, bile, temperature and the best growth properties.

Key words: aquaculture; *Oncorhynchus mykiss*; probiotics; selection criteria

INTRODUCTION

The fact that pathogenic strains, such as *Aeromonas* species, are resistant to a number of antimicrobial agents, suggests caution in the treatment of aquatic animals with antibiotics (Aravena-Román *et al*., 2012). A serious problem of multi-resistance has been demonstrated in several farms (Balta *et al*., 2016). For these reasons, we are looking for new safe solutions using mainly substances of natural origin, which would not reduce the quality of aquaculture products and, at the same time, would not burden the environment. Such an alternative is represented by probiotic microorganisms, by means of which it is possible to modulate not only the intestinal microbiota of aquatic animals in aquaculture, but also the microbiota of the aquatic environment (Newaj-Fyzul *et al*., 2014). In terms of safety for the use of probiotics in practice, the fact that probiotic microorganisms will not increase the already existing risks of antibiotic resistance associated with normal microbiota in the intestine or in food must be confirmed. In the European Union, all microorganisms are subject of antimicrobial susceptibility testing before being used as a feed additive (Bories *et al*., 2008; EFSA, 2012; EFSA, 2013).

Previously, probiotics were used to improve water quality and control bacterial infections. However, there is also documented evidence that probiotics can improve nutrient digestibility, increase stress tolerance and promote reproduction. In the past, aquaculture research has focused on

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known probiotic strains derived from terrestrial animals, ignoring the fundamental differences in the physiology of cultivated species – mammals or birds versus fish – as well as the differences in the environment, where the relevant microbial communities have been developed - aquatic versus ground environment. Therefore, probiotic bacteria isolated from the respective host (fish) are expected to perform better in their natural environment than those originating from terrestrial hosts (Van Doan *et al*., 2018).

The selection of probiotics for aquaculture and their development for commercial use in aquaculture is a multistage and multidisciplinary process requiring first basic and later applied research and assessment of its use in practice (Edun and Akinrotimi, 2011). Anyway, microorganisms used as probiotics must be safe not only for aquatic hosts, but also for their surrounding environment and humans (Muñoz-Atienza *et al*., 2013). Merrifield *et al*. (2010) identified a list of properties that potential probiotic bacteria for aquaculture should meet. The isolates should be subjected to microbiological, biochemical and genetic testing to identify and select the most suitable candidates for further assessment as probiotics for sustainable aquaculture. It is unlikely to find a candidate that meets all the criteria and, therefore, it is necessary to focus research on the concomitant use of several probiotic strains or a symbiotic combination of a probiotic with a prebiotic (Ibrahem, 2015).

An important selection criterion that a candidate probiotic for aquaculture must meet is a survival in the digestive tract. If the probiotics tolerate acidic environment and bile, they will survive the passage through the gastrointestinal tract more easily and may colonize the intestine (Sica *et al*., 2012). Another selection criterion is the determination of antagonistic activity of potential probiotic strains against key fish pathogens. For salmonids, the main pathogens are *Aeromonas salmonicida* subsp. *salmonicida* and *Yersinia rückeri*. The lactobacilli that we have isolated, showed inhibitory activity against both tested pathogens of *Aeromonas salmonicida* subsp. *salmonicida* CCM 1307 and *Yersinia rückeri* CCM 6093. Antagonist activity was determined on the basis of the inhibition zones obtained by performing a combination of the disc diffusion and pouring method (Fečkaninová *et al*., 2019).

In order to confirm the results obtained *in vitro*, these results must be verified in a clinical trial. The results of clinical trials with probiotics are very contradictory. Several authors have described positive preventive or therapeutic effects of probiotics in various diseases of salmonids (Irianto and Austin, 2002; Brunt *et al*., 2007; Maricchiolo *et al*., 2015). However, other reports have not detected any, or significant, effects of probiotic microorganisms on the health status of aquatic animals (Gomez-Gil *et al*., 2000; Marques *et al*., 2004). Scientific studies have shown that probiotics are most effective in animals during the development of their microbiota or when their stability is impaired (Fečkaninová *et al*., 2017). Differences in results are due to many factors, such as probiotic strain selection, survival and stability, species specificity of the strain in relation to the host, the dose, the frequency and route of application of the probiotic, fish health and nutritional status, species and age, drug or microbiota interactions, the stress, the overall organization of the experiment etc. (Fečkaninová *et al*., 2019).

Currently, fish farms are facing a reduced amount of available water and deteriorating water quality in rivers. This can lead to oxygen reduction and accumulation of fish metabolites, ammonia, CO_2, NO_2^- in the water. Impairment of water quality creates stress, increases susceptibility to disease, affects feed intake, growth, and induces a decrease in fish welfare (Ellis *et al*., 2002). Research on the interaction of water quality and welfare should be encouraged, particularly in commercial fish farming conditions (EFSA, 2008). In rainbow trout aquaculture, good water quality and its sufficient quantity are important (Sener, 2012). The decrease in dissolved oxygen concentration in water is a consequence of its consumption by fish and decomposing organic and inorganic substances. Decomposing waste materials are subject to biochemical oxidation associated with the evacuation of oxygen, the intensity of which depends largely on the temperature of the water and the qualitative composition of the decomposed organic matter and its quantity (Pokorný *et al*., 2003).

Feed is considered to be the most important polluting factor in fish farming. The effect of feeding on changes in water quality flowing through a fish farm depends primarily on the composition and physical properties of the feed, the technology of its production, the digestibility and quality of the components of the present feed and the feeding technique (Pokorný *et al*., 2003). For the production of rainbow trout, an energy-rich extruded feed is used, which has a significant effect on the quality of run-off water (Viadero *et al*., 2005). On the other hand, the quality of the inflow water also has a considerable influence on the effluent parameters. Fish feeding is the only factor affecting all measured water parameters (Bergheim and Asgard, 1996). Organic pollution is considered to be a significant negative factor in salmonid fish farming, and the requirements for the lowest possible organic load on water are among the most important. The decomposition of organic matter leads to the depletion of oxygen and the formation of toxic substances (ammonia, hydrogen sulphide, methane and others). There is also an increased risk of serious fish diseases (e.g. bacterial gill disease).

Feeding experiments are costly- and time-consuming, therefore, it is necessary to establish rapid *in vitro* screening strategies to select the most promising isolates. Ideally, *in vitro* screening would allow identification of the beneficial effects and reduce the risk of negative effects. In addition, such research can provide new insights into the biology and ecology of autochthonous bacteria and improve knowledge on microbial host interactions (Wong and Rawls, 2012).

CONCLUSION

It is assumed that probiotic strains can significantly contribute to improving fish health and also improve the immune status of fish. They can also contribute to improving fish production parameters (feed conversion and increasing weight gains), thus contributing to improving the economic viability of farming. The strains are expected to demonstrate a dominant ability to colonize intestinal mucosa of fish even after *in vivo* application. The presence of a dominant bacterial strain at high densities in an aqueous environment indicates its ability to grow successfully under given conditions and it can be expected that this strain will compete effectively for nutrients with possible undesirable microorganisms. In order to confirm this assumption in further research, it will be necessary to prepare a dosage form of selected strains of probiotic bacteria and subsequently verify the *in vitro* properties of the bacteria under *in vivo* conditions. It will be necessary to test the interactions of the strains with the feed components, the aquatic environment and the real digestive tract conditions of the fish.

There is a wealth of information regarding the microbial modulating effects of dietary modifications and the presence of LAB in the gastrointestinal tract (GIT). However, in the screening of the GIT, there is a concern that most studies evaluating microbiota in the intestine focus on characterizing the communities in the GIT (allochthonic microbiota), while those bacteria that have the ability to adhere to the mucosal surface (autochthonous microbiota), which are important for specialized physiological functions, remain uncharacterized. Therefore, we recommend paying more attention to the autochthonic intestinal microbiota.

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