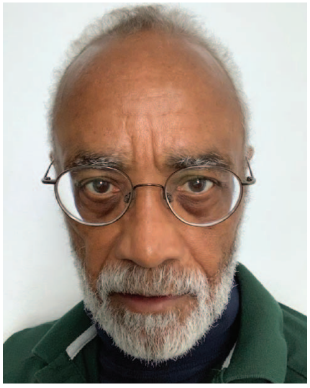
Probiotics for cultured freshwater fish





Javier Fernando Melo-Bolívar[A,B], *Ruth Yolanda Ruiz-Pardo*[A], *Michael E Hume*[C], *Hanna E Sidjabat*[B] *and Luisa Marcela Villamil-Diaz*[A,D]

[A]Universidad de La Sabana, Doctorado en Biociencias, Chía, Colombia

[B]UQ Centre for Clinical Research, The University of Queensland, Brisbane, Qld 4072, Australia

[C]United State Department of Agricultural, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, USA

[D]Email: luisa.villamil@unisabana.edu.co

Abstract. **Probiotic products are viewed as an alternative to the use of antibiotics in freshwater fishes farming. Probiotic organisms include bacteria, yeast, and filamentous fungi offering different benefits to fish including growth promotion, inhibition of pathogen colonisation, and improvement of nutrient digestion, water quality, and stress tolerance, as well as enhancement of reproduction. For these reasons, this review aims to identify the main trends in probiotic amendment in freshwater fishes. Strategies to incorporate the probiotic strains in the fish feed or pellets to allow optimal viability of the strains as they reach the fish gastrointestinal tract (GIT) are crucial in probiotic research and commercial applications for freshwater fish.**

Tilapia dominates the aquaculture industry in many tropical and subtropical countries and is one of the most important protein sources from freshwater fish[1]. Traditionally, antibiotics and chemicals have been used to treat infectious diseases in fish. As an alternative to the use of antibiotics, probiotics originated from the native gastrointestinal microbiota of fish have been increasingly common within the past two decades[2]. Probiotic organisms include bacteria, yeast, and filamentous fungi often originate from the GIT of fish, and can be applied individually or in mixtures or consortia.

In 2017, more than 150 million tons of fish were produced worldwide, with China being the largest producer country with 4 million tons of total product[3]. By 2030, it is expected that close to 62% of consumed fish will come from aquaculture and 38% from wild-caught fish[4]. However, one of the main difficulties in the commercial cultivation of aquatic organisms is the appearance of infectious diseases that hamper industry sustainability. Several researchers and producers point to disease as the leading cause of losses in production and economic resources[5].

Probiotic bacteria give multiple benefits to fish, such as growth promotion, inhibition of pathogen colonisation, and improvement of nutrient digestion, water quality, and stress tolerance, as well as enhancement of reproduction[6]. Yeast is the second group of microorganisms with probiotic potential. Yeast as probiotic supplements in the fish diet offer benefits that include modulation of the digestive microbiota, enhancement of immune responses, contributions to intestinal enzymatic physiology, and enhanced growth performance[7]. The third group of microorganisms with probiotic potential are the filamentous fungi. These fungi stimulate antioxidant response and the immune system, and additionally stimulate the production of various digestive enzymes including amylases, cellulases, β-glucanases, xylanases, proteases, and lipases[8]. Generally, these probiotic microbes are non-fish derived. In contrast, a multi-strain probiotic culture, maintained in continuous culture, was recently developed from Nile tilapia (*Oreochromis niloticus*) gastrointestinal microbiota. A *Cetobacterium* sp. was the dominant genus, and the multi-strain culture had *in vitro* antibacterial activity against fish pathogens such as *Streptococcus agalactiae* and *Aeromonas hydrophila*[9]. Results identified three bacteria within the continuous culture with distinct antibacterial activity against these pathogens.

It is essential to define the probiotic dosage for a specific fish and environment in order to avoid economic losses due to overdose or too low a dosage[10]. Most of the journal articles sourced suggested the use of probiotic concentrations of approximately 1×10^6 CFU/g of feed showed significant improvement in growth performance, resistance to infection, and immune modulation. Growth performance and health status improved in trout[11], and tilapia[12], with a probiotic microbial concentration around 1×10^6 CFU/g of feed. According to Merrifield *et al.*[13] that concentration of probiotics is necessary to ensure the colonisation of the probiotic in the intestinal tract of fish.

A very high concentration of probiotics, exceeding what is optimal, could result in wasted energy and nutrients, and, for that reason, it is necessary to test the functional dosage of probiotics in every particular situation[14]. Farias *et al*.[15] showed that a higher dosage than needed could partially suppress probiotic responses. The lower concentration tested (1×10^7 CFU/g) was enough to offer an improvement in growth performance and nutrient utilisation in *Oncorhynchus mykiss*[11] and *Salmo salar*[10]. Bhujel *et al.*[16] used regression analysis to define the optimal concentration of the two commercial probiotic formulations in *Labeo rohita,* showing that the effective dosages were higher concentrations than that suggested by the manufacturers.

Another critical factor for probiotic efficacy is the administration period. Researchers generally administrated multi-strain probiotics over periods of approximately 30 days (28–30 days), 45 days (42–49 days) and 60 (56–60 days). Addo *et al.*[17] found that growth performance was low at 21 days of treatment with a mixture of two *Bacillus* strains (SB3086 and SB3615). Similarly, *Bacillus subtilis, Saccharomyces cerevisiae* and *Aspergillus oryzae* did not show improvement in growth performance over 28 days of treatment[18]. However, the administration of *Micrococcus* sp. and *Bacillus* sp. enhanced growth performance of *Etropus suratensis* at day 28 in comparison to 14 days of administration. Other authors considered that more than 45 days are needed to confirm the probiotic potential offered by a mixture of probiotics. Giri *et al.*[19] showed that after 60 days, growth performance and immune modulation improved. Similarly, growth parameters improved with the administration of *Enterococcus faecium* and *Geotrichum candidum* to *L. rohita*[20] for 45 days and 90 days. The administration of a probiotic in *Piaractus mesopotamicus* showed an increase in survival and biomass production benefits offered by microorganisms administered over a more extended period[21]. Merrifield *et al.*[13] demonstrated that a continuous administration enhanced colonisation and probiotic activity in the rainbow trout (*O. mykiss*) gastrointestinal tract.

Generally, it is recommended that probiotics be applied in the earlier growth stages. The administration of two types of probiotic mixtures in *L. rohita* at different stages showed that growth performance and survival improved in hatchlings and fry, but administration only in the advanced fry stage did not affect survival and growth[16]. Likewise, Jha *et al.*[22] demonstrated that early administration improved survival and growth of *L. rohita* hatchlings and fry. In contrast, probiotic administration to advanced fry did not cause any effect. Similarly, Ridha *et al.*[23] showed that application of two types of probiotic mixtures improved growth parameters at the juvenile stage, more than when administered at the adult stage. However, the recommendation is to evaluate probiotics applications from earlier stages to market size with continuous administration and treatments being withdrawn at different periods[16].

The method of administration has been shown to affect probiotic performance[24]. The method most commonly used for administering probiotic mixtures is incorporation into the feed (92.8%), followed by direct incorporation into the water (4.8%) and in live food (1.6%). The process for incorporating the probiotic into feed has different stages: mixing the probiotic with feed, adding water, pelletising to the selected size, drying, packaging, labeling, and storing until feeding.

There are a few journal articles in which the authors measured the viability of the bacteria incorporated into the feed. Aly *et al*.[25] centrifuged and washed the probiotics with a buffer, and added a concentration of 1 × 10^9 CFU/g to feed. The feed was blended in an automatic mixer and pelletised. The pellets were dried in an oven at 45°C and the probiotic viability was measured weekly over five weeks of storage at 4°C and 25°C. Results showed that *B. pumilus* survived at both temperatures over the five weeks. Meanwhile, *Citrobacter freundii* and *B. firmus* survived at 4°C during the five weeks, but at 25°C, they were viable for one or two weeks, respectively. In another study, probiotics were centrifuged, and bacterial pellets were resuspended in nutrient broth[26]. The probiotic suspension was sprayed at ~2 × 10^9 CFU/g on feed in plastic trays and air-dried in a microbial cabinet at room temperature (19°C). Viability was measured after three months of storage at 4°C, showing that *Exiguobacterium* JHEb1, *Vibrio* JH1, and *Enterococcus* JHLDc were viable at concentrations of more than 1 × 10^7 CFU/g of feed over the three months evaluated. Finally, *Bacillus amyloliquefaciens* 54A and *B. pumilus* 47B at three different concentrations (1 × 10^9, 3 × 10^9 and 5 × 10^9 CFU/g of feed) were mixed with feed and viability at 4°C was evaluated every week showing that the probiotic level decreased 10% after every three weeks of storage[27]. From the processing perspective to incorporate the fish probiotics, *Bacillus* spp. would be more suitable to be incorporated in the fish feed or pellets, since they are spore-forming bacteria, which allow heat resistance during the pellet compression process[28]. Floating fish feed is more desirable for fish. Therefore, probiotics that survive the preparation process for dry pellet would be more effective as fish feed.

Selection of probiotic strains should consider the potential unexpected transmission of the strains to human, or their genetic characteristics to other bacteria. Therefore, strains identified as probiotics for fish might not necessarily be suitable for commercial probiotics. Strains such as *E. faecium* and *C. freundii* are considered as opportunistic pathogenic bacteria in humans[29, 30]. Therefore, these strains are not recommended for future fish probiotics products.

In summary, probiotic microorganisms are a healthy, sustainable, and environmentally friendly approach in comparison with antibiotics to reduce the loss of aquaculture fish in disease outbreaks. Probiotics offer several benefits, including improved growth, immune modulation, and disease resistance. However, it is important to define the proper dosage, the administration period, fish stage at administration, administration method, and probiotic viability during production and storage in order to offer optimal probiotic efficacy in aquaculture fish. At the same time, it is important to evaluate its safety application on humans, other animals, and the environment.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Javier Melo is a PhD student working on the evaluation of the probiotic potential of a competitive exclusion culture of the intestinal microbiota of tilapia (*Oreochromis niloticus*). Javier Melo has a Master's degree in Process Design and Management from the Universidad de La Sabana, where he studied the microencapsulation of probiotic spores and their inclusion in juices and dairy products. Javier has a degree in Medical Laboratory Science from the Universidad Colegio Mayor de Cundinamarca in Colombia.

Dr Ruth Ruiz is a Chemical Engineer from the Universidad Nacional de Colombia. Dr Ruth Ruiz has a Master's degree in Industrial Engineering from the Universidad de Los Andes. Dr Ruth has a PhD in Engineering where she studied the re-use of syrups from osmotic dehydration of fruits – cryoconcentration of high initial concentration sucrose solutions at the National University of Colombia. Dr Ruth Ruiz is an expert in industrial processes that include cryoconcentration, lyophilisation and production of microorganisms in bioreactors.

Michael Hume has a BS and MS from the Virginia Commonwealth University and a PhD from Oklahoma State University. His doctoral research investigated fluid transport across salivary gland membranes of the lone star tick (*Amblyomma americanum*) as affected by dopamine stimulation of the adenylate cyclase cytoplasmic membrane transport system. Dr Hume has over 30 years of service with the USDA, Agricultural Service investigating the development, discovery, and application of probiotics, prebiotics, and alternatives to antibiotics as dietary supplements to enhance the digestive microbiome to promote protection against invading human enteropathogens and to promote growth and production in food animals.

Dr Sidjabat is an expert in antimicrobial-resistant bacteria and probiotic development. Dr Sidjabat is currently an Adjunct Research Fellow within Menzies Health Institute Queensland, Griffith University, Gold Coast Campus. Prior to her Griffith University affiliation, Dr Sidjabat was a Researcher at the Infectious Diseases Theme at the University of Queensland Centre for Clinical Research (UQCCR) for 10 years working on antimicrobial resistance and probiotic development. Sidjabat's probiotic research work is within the agreement by Uniquest (https://uniquest.com.au/) with probiotic companies. Dr Sidjabat worked as Postdoctoral Research Fellow between December 2007 and 31 May 2009, at the Division of Infectious Diseases, University of Pittsburgh, Pennsylvania, USA. Dr Sidjabat's primary interest in probiotic screening and development is through phenotypic, genomic and proteomic approaches.

Dr Luisa Villamil is a Marine Biologist from Universidad Jorge Tadeo Lozano in Colombia. Dr Luisa Villamil has an MS and a PhD in Biological Sciences and Aquaculture from the Universidad de Vigo in Spain in the application of lactic acid bacteria in turbot (*Scophthalmus maximus*). Dr Luisa Villamil was a visiting Professor in the University of Rhode Island and the Woods Hole Oceanographic Institute. Dr Villamil's main activities are the development of new alternatives to antibiotics and promoting the growth and health of aquaculture organisms.