

Isolation and Probiotic Characterization of *Lactobacillus plantarum* and *Lactococcus lactis* from Gut Microbiome of Rohu (*Labeo rohita*)

Authors : Prem Kumar, Anuj Tyagi, Harsh Panwar, Vaneet Inder Kaur

Abstract : Though aquaculture started as an occupation for poor and weak farmers for livelihood, it has now acquired the shape of one of the biggest industry to grow live protein in the form of aquatic organisms. Industrialization of the aquaculture sector has led to intensification resulting in stress on aquatic organisms and frequent disease outbreaks leading to huge economic impacts. Indiscriminate use of antibiotics as growth promoter and prophylactic agent in aquaculture has resulted in rapid emergence and spread of antibiotic resistance in bacterial pathogens. Over the past few years, use of probiotics (as an alternative of antibiotics) in aquaculture has gained attention due to their immunostimulant and growth promoting properties. It has now well known that after administration, a probiotic bacterium has to compete and establish itself against native microbiota to show its eventual beneficial properties. Due to their non-fish origin, commercial probiotics sometimes may display poor probiotic functionalities and antagonistic effects. Thus, isolation and characterization of probiotic bacteria from same fish host is very much necessary. In this study, attempts were made to isolate potent probiotic lactic acid bacteria (LAB) from intestinal microflora of rohu fish. Twenty-five experimental rohu fishes (mean weight 400 ± 20 gm, mean standard length 20 ± 3 cm) were used in the study to collect fish gut after dissection in a sterile condition. A total of 150 tentative LAB isolates from selective agar media (de Man-Rogosa-Sharpe (MRS)) were screened for their antimicrobial activity against *Aeromonas hydrophila* and *Micrococcus leuteus*. A total of 17 isolates, identified as *Lactobacillus plantarum* and *Lactococcus lactis*, identified by biochemical tests and PCR amplification and sequencing of 16S rRNA gene fragment, displayed promising antimicrobial activity against both the pathogens. Two isolates from each species (FLB1, FLB2 from *L. plantarum*; and FLC1, FLC2 from *L. lactis*) were subjected to downstream probiotic potential characterization. These isolates were compared in vitro for their hemolytic activity, acid and bile tolerance for growth kinetics, auto-aggregation, cell-surface hydrophobicity against xylene, and chloroform, tolerance to phenol, cell adhesion, and safety parameters (by intraperitoneal and intramuscular injections). None of the tested isolates showed any hemolytic activity indicating their potential safety. Moreover, these isolates were tolerant to 0.3% bile (75-82% survival), phenol stress (96-99% survival) with 100% viability at pH 3 over a period of 3 h. Antibiotic sensitivity test revealed that all the tested LAB isolates were resistant to vancomycin, gentamicin, streptomycin, and erythromycin and sensitive to Erythromycin, Chloramphenicol, Ampicillin, Trimethoprim, and Nitrofurantoin. Tetracycline resistance was found in *L. plantarum* (FLB1 and FLB2 isolates), whereas *L. lactis* were susceptible to it. Intramuscular and intraperitoneal challenges to fingerlings of rohu fish (5 ± 1 gm weight) with FLB1 showed no pathogenicity and occurrence of disease symptoms in fishes over an observation period of 7 days. The results revealed FLB1 as a potential probiotic candidate for aquaculture application among other isolates.

Keywords : aquaculture, *Lactobacillus plantarum*, *Lactococcus lactis*, probiotics

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