Aquaculture Reports 30 (2023) 101615

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Evaluation of probiotic potentiality of GM-Lac (*Lactobacillus* and *Bifidobacterium*) in juvenile Asian seabass *Lates calcarifer*

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ARTICLE INFO

Keywords:
Lactic acid bacteria (LAB)
Antimicrobial activity
Lates calcarifer
Growth performance
Gastrointestinal microbiota
Muscle amino acid

ABSTRACT

This study was conducted to evaluate the probiotic potentiality of GM-Lac (a mixture that comprises four lactic acid bacteria (LAB) strains– *Limosilactobacillus fermentum* GMNL-93, *Lactiplantibacillus plantarum* GMNL-141, *Lacticaseibacillus casei* GMNL-277, and *Bifidobacterium lactis* GMNL-550) applied in fish aquaculture. First, the four LAB isolates (GMNL-93, −141, −277, and −550) constituting GM-Lac were tested individually for antimicrobial activity in vitro. Then, GM-Lac was incorporated in fish feed at a dose of 5×10^6 cfu (g feed)$^{-1}$ and fed to Asian seabass (*Lates calcarifer*) juveniles for eight weeks to assess the effects on growth performance, gastrointestinal (GI) histology and microflora, muscle amino acid composition, and disease resistance. According to the result of the zone of inhibition test, each GMNL strain presented noticeable antimicrobial activity against bacterial pathogens common in aquaculture. After the 8-week feeding trial, the fish in the GM-Lac group had higher final weight ($p < 0.05$), percent weight gain, specific growth rate, and feed intake than the fish in the Ctrl group, along with higher microvillus length in GI tract ($p < 0.05$) and greater total amino acid concentration in muscle. Furthermore, the relative abundance of microbial phyla in the GI tract of the fish fed with GM-Lac changed significantly, accompanied by increasing probiotic bacteria and decreasing pathogenic bacteria in the intestine and pyloric caeca. In the challenge test, the fish receiving GM-Lac all survived without any clinical symptoms when challenged with *Streptococcus iniae*. This study concludes that GM-Lac (the combination of *L. fermentum* GMNL-93, *L. plantarum* GMNL-141, *L. casei* GMNL-277, and *B. lactis* GMNL-550) is recommended as a probiotic supplement with high potential in aquaculture practice, which has antimicrobial activities against aquatic pathogens and benefits in enhancing growth performance, intestinal health, muscle amino acid composition, and disease resistance in juvenile Asian seabass.

1. Introduction

The aquaculture industry is expanding sustainably and on the way to meeting the growing demand for aquatic foods (FAO, 2022). Over the past few decades, antibiotics and chemical compounds have been conventionally used in intensive and commercial aquaculture production for getting better growth and disease management. Unfortunately, the misuse of antibiotics may lead to widespread antibiotic resistance, while chemicals may remain in aquaculture ponds and cause environmental pollution (Done et al., 2015). With growing public concerns about food safety and environmental issues, it is urgent to find ways to maintain the health of farmed aquatic animals and reduce the use of antibiotics and other chemicals in aquaculture (Assefa and Abunna, 2018; Marshall and Levy, 2011; Pérez-Sánchez et al., 2018; Silva et al., 2020; Subasinghe, 2005).

Probiotics, such as genera *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Enterococcus*, *Bacillus*, *Escherichia*, and yeast *Saccharomyces*, etc., are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (El-Saadony et al., 2021; Elshaghabee et al., 2017; FAO/WHO, 2002; Fijan, 2014). Many of them have been demonstrated to improve host health by maintaining the balance of gastrointestinal (GI) microbiota and regulating immune responses, recommended as eco-friendly antimicrobial therapies for preventing or treating diseases (Brown et al., 2012; Silva et al., 2020; Suvorov, 2013; Yan and Polk, 2011). Among the probiotic bacterial species, lactic acid bacteria (LAB), represented by the genera *Lactobacillus* and *Bifidobacterium*, are granted GRAS (generally regarded as safe)-status and are one of the most commonly used

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https://doi.org/10.1016/j.aqrep.2023.101615
Received 15 February 2023; Received in revised form 7 April 2023; Accepted 2 June 2023
Available online 6 June 2023
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