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Protection of rainbow trout (*Oncorhynchus mykiss*) from lactococcosis by probiotic bacteria

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Abstract

We analysed the effect of probiotic supplementation on the control of lactococcosis in rainbow trout. Probiotic strains *Leuconostoc mesenteroides* CLFP 196 and *Lactobacillus plantarum* CLFP 238 were administered orally to fish for 30 days at 10^7 CFU g^{-1} feed. Thirty days after the start of the probiotic feeding, fish were challenged with *Lactococcus garvieae*. Probiotic supplementation reduced fish mortality significantly, from 78% in the control group to 46–54% in the probiotic groups.
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Keywords: *Leuconostoc mesenteroides*; *Lactobacillus plantarum*; Probiotics; Rainbow trout; Control; *Lactococcus garvieae*

Résumé

Nous avons étudié l'effet de l'apport des bactéries probiotiques pour le contrôle de la lactococcose chez la truite arc-en-ciel. Les souches probiotiques *Leuconostoc mesenteroides* CLFP 196 et *Lactobacillus plantarum* CLFP 238 ont été fournies à un groupe de poissons à une concentration de 10^7 UCF g^{-1} d'aliment pendant 30 jours. Après cette période, les poissons ont étés infectés expérimentalement par une souche de *Lactococcus garvieae*. Le taux

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de mortalité dans le groupe de poissons qui a reçu le supplément probiotique, a été significativement réduit (78% dans le groupe témoin et 46–54% dans le groupe probiotique).
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Mots clés: *Leuconostoc mesenteroides*; *Lactobacillus plantarum*; Probiotiques; Truite arc-en-ciel; *Lactococcus garvieae*

1. Introduction

Lactococcus garvieae is an important Gram-positive coccus pathogen that causes serious economic losses in cultured marine and freshwater fish species, particularly during the summer months, given its association with high water temperatures [1]. Moreover, in several cases, this microorganism has also been isolated from humans, suggesting that *L. garvieae* could be catalogued as a potential zoonotic agent [1,2]. In fish farming, outbreaks are treated with antibiotics; however, they are often ineffective and their indiscriminate use has led to an increase in antibiotic resistance, particularly drug resistance arising in microorganisms through adaptation or by genetic exchange [3]. Therefore, research on probiotics for aquatic animals is increasing with the demand for environmentally friendly aquaculture [4,5].

Probiotics are defined as live microorganisms that confer a health benefit on the host when consumed in adequate amounts [6]. They favourably influence both the development and the stability of the host's normal microbiota, inhibit colonisation by pathogens, influence the mucosal barrier by their trophic effect on intestinal epithelium and stimulate both specific and nonspecific components of the immune system [7–10].

Studies by our research group have recently demonstrated that *Leuconostoc mesenteroides* CLFP 196 and *Lactobacillus plantarum* CLFP 238 isolated from the intestine of healthy salmonids, exhibit high adhesion ability to intestinal mucus and competitive exclusion of fish pathogens under *in vitro* conditions [11]. The aim of the present study, therefore, was to investigate the probiotic effect of *Leuc. mesenteroides* CLFP 196 and *Lb. plantarum* CLFP 238 in rainbow trout challenged with *L. garvieae*.

2. Materials and methods

2.1. Bacterial strains

Two bacterial strains, *Leuc. mesenteroides* CLFP 196 and *Lb. plantarum* CLFP 238 isolated from salmonids and genetically identified by 16S rRNA gene sequencing, were selected from a pool of 246 strains obtained from intestinal content of healthy salmonids, because their *in vitro* characteristics suggested that they could be considered as potential fish probiotics [11,12]. They were grown aerobically in de Man, Rogosa and Sharpe broth (MRS, Pronadisa, Madrid, Spain) at 22 °C. Stock cultures stored at −80 °C were prepared from overnight cultures

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