Shewanella putrefaciens Pdp11 extracts protect against betanodavirus infection

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Introduction

Viral encephalopathy and retinopathy is caused by the nervous necrosis virus (NNV, *Betanodavirus* genus), a naked virus composed of two single-stranded, positive-sense RNA segments. Betanodaviruses are classified into four species, being RGNNV highly predominant in the Mediterranean area. RGNNV causes high mortality in several fish species, including European seabass. In fact, there are two commercialized vaccines designed to protect seabass against RGNNV infection. In this regard, the development of strategies able to protect different fish species against different viruses, such as the use of probiotics, is a key issue for the aquaculture industry. *Shewanella putrefaciens* Pdp11, SpPdp11, is a fish probiotic with proven positive effects on gilthead seabream and Senegalese sole, protecting those species against bacterial pathogens; however, its antiviral activity is unknown. This study is a step forward in the use of probiotics against viral infections, evaluating the anti-RGNNV activity of sonicated-SpPdp11 extracts *in vitro* and *in vivo*.

Material and Methods

The in vitro evaluation was performed on E-11 cells following three assays: (i) neutralization, (ii) 6-h pre-adsorption, and (iii) post-adsorption, determining the inhibition percentage of RGNNV-induced CPEs and quantifying viral replication. The immunostimulatory activity of SpPdp11 extracts was also examined, analysing the transcription of *mx, hsp70, tnfα, e3* and *tlr3* in E-11 cells.
For the in vivo evaluation, two European seabass groups were established: (i) control group, receiving commercial feed, (ii) experimental group, fed with commercial pellet supplemented with SpPdp11 extracts. Animals were fed for 30 days and subsequently challenged by intramuscular injection. Results were expressed as cumulative survival.

Results

SpPdp11 extracts compromised RGNNV replication in E-11 cells (67.3% and 55% CPE inhibition in 6-h pre-adsorption and post-adsorption assays, respectively), and modulated the transcription of all the E11 immune-related genes examined. The highest induction was obtained for mx gene.
Regarding the in vivo results, 82% of fish fed with the SpPdp11-supplemented diet survived to RGNNV infection, whereas the survival rate of fish fed with the control diet was 64%. These results suggest that SpPdp11-supplemented feed can be a promising prophylactic tool against RGNNV infection.

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