Effects of probiotic *Shewanella putrefaciens Pdp11* on *Solea senegalensis* infected with *Vibrio harveyi*

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Introduction

Spain was pioneer in the cultivation of Senegalese sole (*Solea senegalensis*), making this specie an alternative to traditional farming, thanks to acceptance by consumers, price and demand in our markets. Therefore, a study to improve the production of sole could provide business opportunities.

The use of *Shewanella putrefaciens Pdp11* on cultivated specimens of *Solea senegalensis* has been shown to have a marked probiotic effect, being involved in improvements on growth and immune response (Vidal et al., 2016). The gut microbiota has been considered as one of the most important factors influencing host metabolism, glucose and lipid metabolism, fat distribution and growth performance. The ability of probiotics to colonize the intestine changes the intestinal microbiota and therefore the host's metabolism (Falcinelli et al., 2015).

Vibriosis has become the economically most important disease in marine fish culture, affecting a large number of species. It is a disease caused by pathogenic *Vibrio*, which causes internal and external bleeding in the host. In many cases, strains of the genus *Vibrio* are opportunists, infecting when the host organism is immune suppressed or stressed, with the frequency of infection often being attributable to intensive culture and adverse environmental conditions.

Vibrio harveyi Lg 16.00 causes internal and external hemorrhages, with great affectation in the liver and kidney, in specimens of S. senegalensis. Previous work suggests that one of its pathways of entry of the pathogen could be intestinal and that the administration of the probiotic *Pdp11* significantly decreased the mortality of individuals affected by this pathogen (Tapia-Paniagua et al., 2014).

In this research, the probiotic ability of *Pdp11* to interfere in the oral transmission produced by *V. harveyi Lg 16.00* in *S. senegalensis* is studied. To this end, the objectives are: (1) To observe variations in the glucose metabolism in liver and kidney, by fluorodeoxyglucose (18-FDG); (2) histology and histomorphology study of liver; (3) variation of lipids in the intestine and intestinal content.

Materials and methods

Four groups of *S. senegalensis* (1.2-1.5 kg) were tested (1) control, with a commercial feed diet; (2) control + vibrio, fed with a commercial diet and received a single oral dose (1ml/kg) of the suspension of *V. harveyi Lg 16.00* (10^8 cells/ml); (3) *Pdp11*, fed with commercial diet and oral suspension of 1ml/kg of the probiotic *Pdp11* (10^8 cells/ml) (4) *Pdp11* + Vibrio, which was fed with the diet of the *Pdp11* group and with a single

oral dose (1ml/kg) of the suspension of *V. harveyi Lg16.00*. All the specimens were fed every 8 hours with the detailed diets during a period of 6 days; the infection was carried out 18h before sampling.

18–FDG marking: After infection with the pathogen, all specimens received intraperitoneally fluorodeoxyglucose (18-FDG), (150 uCi/kg). The specimens were kept in the tanks for 30 minutes to assure the distribution of the radiopharmaceutical by the organism. After sacrifice them, they were analysed by positron emission tomography (PET) in the facilities of the Molecular Imaging Unit (CIMES-UMA).

Liver histology and histomorphology: Liver samples were fixed in 4% paraformaldehyde and included in Paraplast. The sections were obtained with the help of a microtome and were stained with hematoxylin-eosin following the routine methodology. Complete images of the stained sections were digitized and 3 captures were studied at 1000x of each one. The lipid area of the liver was measured using ImageJ Freeware (version 1.51) as described in Safer (2017). In this way, the total percentage of lipids present in the liver of the specimens studied was calculated.

Intestinal lipids: The lipid content was measured after extraction with chloroform / methanol (2:1 v/v) according to the method described by Folch et al. (1957). The lipids were dissolved in toluene and the fatty acid methyl esters (FAME) were obtained by transesterification with sulfuric acid (1%) in methanol.

Statistical differences were determined using t-test; P-values <0.05 were considered significant.

Results

The results obtained showed a significative increase in the concentration of the radiolabel in the liver and kidney of the control infected with pathogens, in contrast to those who received the probiotic. This result indicates a higher metabolic rate in the organs of individuals affected by the pathogenic strain. This decrease in marked glucose indicates that the probiotic *Pdp11* interferes with the pathogenic process of *Vibrio harveyi Lg 16.00*.

Regarding the histological samples, the lipid area (μm^2) were studied in the hepatocytes of the different treatments. Although the results obtained were not significant, due largely to the heterogeneity of the samples, it was observed that in livers of the Pdp11 + Vibrio group there was a tendency to decrease the lipid area (Figure 1).

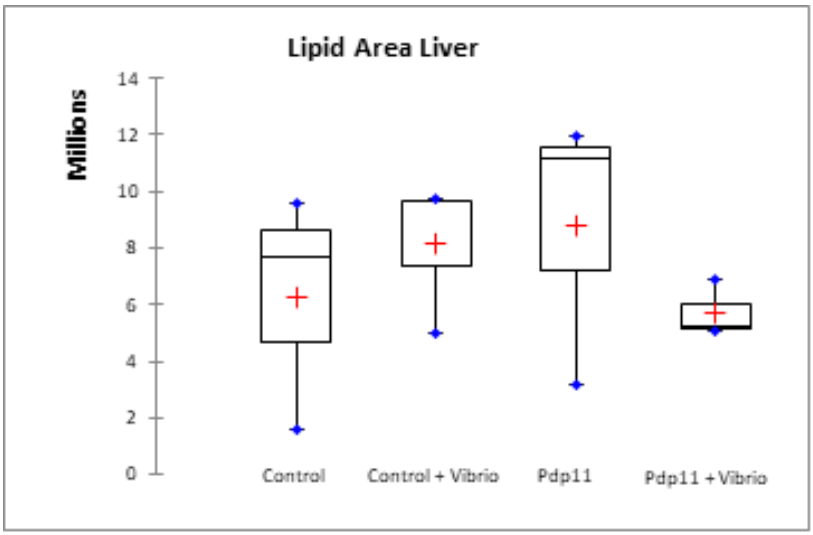

Figure 1: Box plot of lipid area from liver tissue of each treatment.

A decrease in the lipid content in the liver of the Pdp11 + Vibrio group could be related to the energetic need of the organism against infection. The ability of the probiotic to activate the immune system makes the response faster and more effective; this can cause an increase in the metabolism of the fish that has depleted the glucose reserves, the lipids will be used for energy.

Finally, preliminary results on the amount of lipids in the intestine and the intestinal content of different diets, does not show significant differences. However, it is necessary to compare how intestinal lipid change with the presence of *V. harveyi Lg 16.00*.

Acknowledgments

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