Study of the intestinal microbiota of *Solea senegalensis* specimens after the administration of the probiotic *Shewanella putrefaciens* SpPdp11 by Next Generation Sequencing

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Keywords: Aquaculture, Probiotic, Microbiota, Solea senegalensis, Shewanella putrefaciens

Introduction

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Araya et al., 2002). The use of probiotics is a key tool to protect farmed fish, in many cases predisposed to stress and/or infection under intensive culture conditions. In this way, *Shewanella putrefaciens* Ppd11 (SpPdp11) is a microorganism applied to farmed fish such as *Solea senegalensis* and *Sparus aurata* that has demonstrated probiotic effect such as promotes the growth and a better efficiency of feed utilization, stimulating the immune system of *S. senegalensis* and *S. aurata*, and the stress tolerance of *S. senegalensis* specimens to high stocking densities (Tapia-Paniagua et al., 2014). In addition, its capability to modulate the intestinal microbiota of these farmed fish has also been demonstrated using Denaturing Gradient Gel Electrophoresis (DGGE). At present, the Next Generation Sequencing (NGS) methodology is a better and more sensitive way to evaluate the composition of the microbiota and to analyze the effects on it of different factors, such as the dietary supplementation with a probiotic.

In this context, this is the first time that the effect of the probiotic on the intestinal microbiota of *S. senegalensis* is analyzed using the NGS methodology.

Materials and methods

SpPdp11 cells were cultured following the methodology previously described by Tapia-Paniagua et al Tapia-Paniagua et al. (2014). The commercial pellet diet LE Europa GR2 (16 % total lipids and 57% crude protein, Skretting, Spain) was used as control (diet C). The same diet was supplemented with SpPdp11 cells following the methodology described by Tapia-Paniagua et al. Vidal et al. (2016) (diet AP).

Specimens farmed Senegalese sole juveniles (30 - 5 mean weight) from the Spanish Institute of Oceanography (Santander, Spain) were acclimated for 2 weeks prior to the experimental period. Then, fish were randomly distributed in two tanks by diet. The weight of the fish was measured at 0, 15, 30, and 45 days of feeding. Fish from each group were fed 8 times a day for 45 days with the corresponding diet. Three fish of each tank were sacrificed and whole intestines were obtained. Fragments of 0.5 cm of the anterior and posterior intestine were collected and stored at -80 C for intestinal microbiota analysis.

DNA extraction was carried out following the methodology previously described by Tapia-Paniagua et al. (2010). DNA were sequencing by Chunlab Inc. (Seoul, South Korea). Bioinformatic flow was generated by the open source software package MOTHUR (version 1.3), reads were analysed by Greengenes (version 2013)

and statistical analysis were analysed by R Software and open source software online Microbiome Analyst after all random subsampling was conducted to normalize the data size to 7200 reads.

Results and discussion

In comparison with the fish fed the control diet, the growth was higher in fish fed the AP diet at 15 and 30 and significantly higher at 45 days .

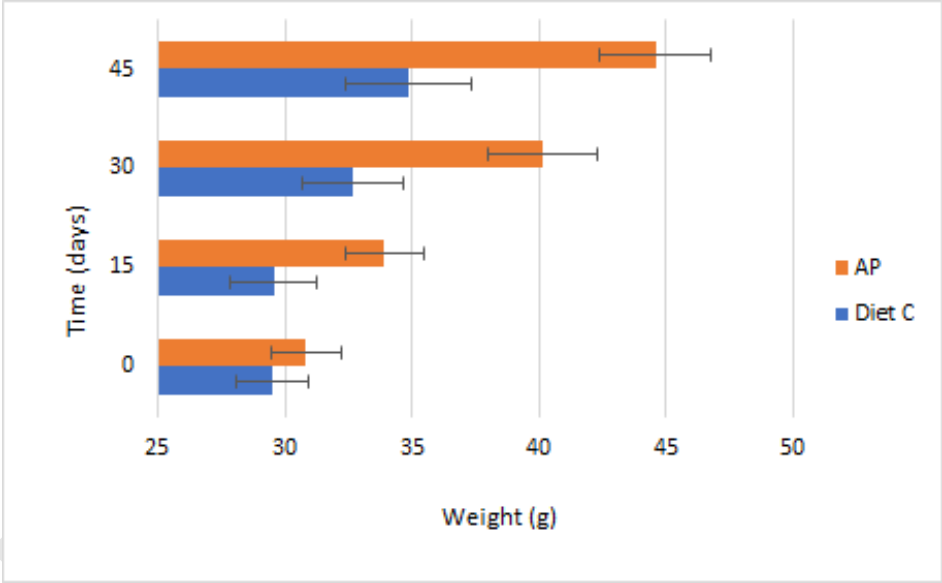

Figure 1: Weight rate (g) of S. Senegalensis receiving during 45 days the control diet (Diet C) and the probiotic diet (Diet AP

In total, 319174 raw reads were obtained for both forward and reverse directions after sequencing. The mean read depth per sample was 26597 - 3223,6 (mean - SD) sequences per red direction. Singletones were removed and a total of 599 OTUs at 97% gene similarity cut off were obtained.

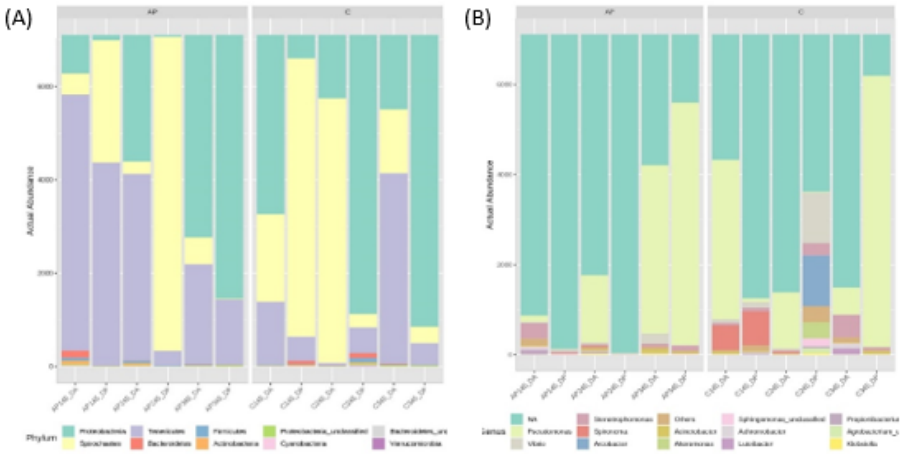

Figure 2: Composition of intestinal microbiota at phylum (A) and genus (B) level in the anterior (DA) and posterior (DP) intestine of specimens of Solea senegalensis fed a control diet (diet C) and the probiotic diet (diet AP)

Results from taxonomical analysis showed that, C and AP diets had a similar composition of intestinal microbiota. The most representative phyla were Proteobacteria, Tenericutes and Spirochaetes. AP diet increased abundance of Tenericutes in constrast with Spirochaetes 2.

At genus level, results showed a considerable presence of *Pseudomonas* in both diets and fragments of intestine. Others representative genus were *Propionibacterium*, *Spironema*, *Stenotrophomonas*, *Vibrio*, *Arcobacter* and *Achromobacter*. It seems to be a higher presence of *Spironema* in C diet than AP diet 2.

This study present Next generation sequencing (NGS) for studying the microbiota, so we can observe microbial variability between treatments, even non cultivable microorganisms or very poorly represented microorganisms.

In general, genera observed, such as *Stenotrophomonas*, *Vibrio* and *Spironema* have been previously reported as intestinal predominant in *S. senegalensis* (Tapia-Paniagua et al., 2014).

Pseudomonas have been described because of interacting positively with epithelial cells in the intestinal mucosa and exerting an important role like antagonist in salmonids. A positive feature is the presence of *Propionibacterium* which species seems to reduce the antinutritional effects of lectins and exert anti-inflammatory properties in mixtures with species of *Lactobacillus*.

In addition, the administration of *S. putrefaciens* like symbiotic with sodium alginate confer a form of synergism, enhancing beneficial effects of the probiotic and a better growth of fish due to an improvement on feed utilization.

Acknowledgments

This study had the financial support of MINECO (Grant no. AGL2014-51839-C5-2-R and AGL2017-83370-C3-3-R) and by European Regional Development Funds.

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