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MICROBIOME NETWORK ANALYSIS IN SKIN AND GILLS OF SPARUS AURATA FED WITH NANNOCLOROPSIS GADITANA MICROALGAE

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Introduction

One of the major current challenges of aquaculture industry involves the development of new commercial diets with the aim of reducing the unsustainable consumption of fishmeal while improving fish nutrition. In this context, *Nannochloropsis gaditana* is a microalgae rich in beneficial lipids, antioxidant compounds and amino acids, which has demonstrated improvements in growth and product quality in juvenile *Sparus aurata* (Ayala et al. 2020). In that sense, external barriers such as skin and gills can be used to measure how diets can affect and modulate the animal's metabolism. Both tissues are covered by a mucus secretion that includes a high number of microorganisms and molecular components of the immune system. The aim of the work is to observe what changes occur at the microbiota level in the skin and gills of commercial-sized *S. aurata* fed with a diet supplemented with raw and hydrolysed *N. gaditana* microalgae for 90 days.

Material and methods

N. gaditana biomass was produced in closed tubular photobioreactors at the Estación Experimental de las Palmerillas de la Fundación Cajamar following the standard procedure. Fresh *N. gaditana* was hydrolysed using commercial enzymes with cellulase activity (Vizcozyme®). The experimental feed was formulated by the Experimental Feed Service of the University of Almeria. The feeding trial was carried out at the facilities of the Central Service for Marine Culture Research of the University of Cadiz, where juvenile *Sparus aurata* weighing 10-15g were divided into 400L tanks and fed for 90 days with the study diets: a commercial diet (Control), a diet supplemented with hydrolysate and crude extract of *N.gaditana* at 5% (Hydrolysate and Crude, respectively). After 90 days, fish were anaesthetised and dissected for further analysis.

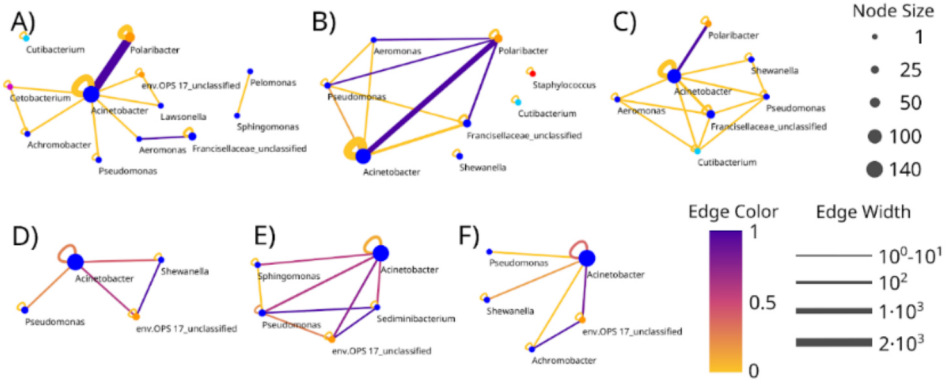

Figure 1. Genera involved in co-occurrence networks. Control diet (A), raw diet (B) and hydrolyzed diet (C) samples of gills. Control diet (D), raw diet (E) and hydrolyzed diet (F) samples of skin. The colour of the interactions is marked by the proportion of negative interactions.

(Continued on next page)

Skin and gill DNA was extracted according to Martinez et al., (1998). The 16S rRNA gene of the samples was sequenced using the Illumina® MiSeq platform (Illumina, San Diego, CA, USA) by pairwise sequencing (2 × 300 bp) at the Ultrasequencing Service of the Bioinnovation Centre of the University of Malaga (Malaga, Spain). Data processing was performed bioinformatically using a workflow based on the DADA2 library of R, using the SILVA 138 database with 99% clustering. Results were considered significant between Shannon index if t-student's $p<0.05$; while taxonomic comparison was performed using the R package DESeq2 ($p<0.05$). Co-occurrence networks were inferred from ASV abundances, based on Spearman correlation with the Hmisc library in R. Only Spearman correlations $p<0.01$ and with $\rho>|0.60|$ were considered statistically significant (Barberán, 2012) and visualised in Cytoscape 3.9.1. Only the ASVs present in more than half of the samples for each diet and tissue were considered in this network analysis.

Results and discussion

1416 and 694 ASVs in gills and skin respectively were preserved in the taxonomic analysis after filtering. The predominant *phylum* in gills was Proteobacteria (~50%) in the control and hydrolyzed groups and reached 70.58% in the raw diet. The Bacteroidota *phylum* was the most represented and Firmicutes and Actinobacteria were close to 3% in all treatments in this tissue. However, in all three different treatments, the phyla abundance was similar in skin samples, ~90% of which corresponded to Proteobacteria. Bateroidota (5.5%–5.9%), Firmicutes (1.8%–2%) and Actinobacteria (<1%) constitute the other phyla. Significant differences between treatments for each tissue were calculated. In gills, 13 significantly higher ASVs were obtained in the control (such as *Achromobacter*, *Acidobacter*) versus 4 whose abundance was higher in the hydrolyzed diet. The number of ASVs that differed significantly between the gill microbiota in the control vs. raw group amounted to 70, most of them being ASVs corresponding to the genus *Shewanella* (43) higher in the raw diet. Nevertheless, in the skin samples, Control group showed a significant increase of abundance related to *Acinetobacter*, *Achromobacter*, *Pseudomonas*, *Shewanella*, *Vibrio* and *Sphingomonas* among others. In the hydrolyzed group, the most significant abundance was associated with the genus *Pseudomonas*, *Vibrio*, *Pseudoalteromonas*, *Ralstonia* o *Cutibacterium*. In the case of the skin raw samples, there were ASVs corresponding to the genus *Acinetobacter*, *Streptococcus* or *Pseudoalteromonas* that were significantly different respect of control.

Taking the ASV abundance matrix for each diet and tissue, 6 co-occurrence networks were constructed. In all treatments in gills, *Acinetobacter* was a central genus in the network, and exhibited a negative correlation with *Polaribacter*. Besides, in the raw group, *Polaribacter* also showed negative correlation with *Aeromonas, Pseudomonas* and *Francisellacea*. In skin, *Acinetobacter* was involved in a major proportion of co-exclusion relations interacting with *Acinetobacter, Sphingomonas, Pseudomonas, Sediminibacterium* and env.OPS17 (Figure 1). This work shows that *Acinetobacter* has a key role in the balance of mucosa microbiota and was in co-exclusion with *Polaribacter*

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