EVALUATING *Lactococcus lactis* STRAIN AS PROBIOTICS FOR GILTHEAD SEA BREAM (*SPARUS AURATA*): EFFECTS ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY, TRANSCRIPTIONAL RESPONSE AND GUT MICROBIOTA

F. Moroni[1]*, F. Naya-Català[2], M. C. Piazzon[3], S. Rimoldi[1], J. Calduch-Giner[2], F. Brambilla[4], J. Pérez-Sánchez[2] and G. Terova[1].

[1]Department of Biotechnology and Life Sciences, University of Insubria, Via J.H. Dunant, 3, 21100 Varese, Italy.
[2]Nutrigenomics and Fish Growth Endocrinology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain.
[3]Fish Pathology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain.
[4]VRM S.r.l. Naturalleva, Via S. Michele,41 - 37044 Cologna Veneta (Verona), Italy.
E-mail: f.moroni@uninsubria.it

Introduction

In aquaculture a great number of bacterial species are used as probiotics (Newaj-Fyzul et al., 2014). The use of these microorganisms is mostly related to the need to decrease or avoid the use of antibiotics, increasing the sustainability of the aquaculture industry. Probiotics can reduce pathogenic bacteria due to direct competition dynamics, producing inhibitory molecules and enhancing the host immune system (Balcázar et al., 2007). In cultured fish, probiotics improve fish growth and feed conversion rates, due to an increase in feed digestibility and absorption of nutrients (Merrifield et al., 2010; Martínez Cruz et al., 2012). Furthermore, the use of probiotics can restore the eubiotic state of the intestinal microbiota or can help maintain gut microbiota homeostasis (Borch et al., 2015; Ringø et al., 2016). Accordingly, the aim of the present research was to evaluate, in gilthead sea bream (*Sparus aurata*), the effects of the administration of the probiotic *Lactococcus lactis* subsp. *lactis* SL242, on growth performance, feed utilization, intestinal morphology, transcriptional response, and gut microbiota.

Materials and Methods

The trial was conducted with sea bream juveniles (70-90 g), individually tagged and reared in nine 500-L tanks with 40 fish/tank. Fish were divided into 3 groups and were fed with a control (diet A) or experimental diets (diets B and C), supplemented with 2.0 E+09 CFU/kg and 5.0 E+09 CFU/kg dose of probiotic, respectively. At the end of the feeding trial, all the animals were weighed, in order to calculate the growth index, and four fish per replicate were sacrificed to collect intestinal samples for morpho-histological evaluation, gene expression and microbiota analysis. The latter performed using Illumina MiSeq platform and a metagenomics pipeline based on VSEARCH and RDP databases.

Results and Discussion

The final biomass of fish fed diet C was significantly higher than the control group (diet A), with intermediate values for fish fed diet B. Indeed, even if without significant differences, the best growth performance tended to be achieved by the animals that have received the higher dose of probiotic. Histological analysis, performed using a quantitative metric system, confirmed that probiotic did not alter the macroscopic morphology of the intestine and did not cause inflammation. In addition, the microscopic evaluation detected an amelioration of the intestine structure, through the increase of the height of the mucosal folds and the number of goblet cells, in fish fed probiotic. These results suggest that *L.lactis* SL242 played an important role improving the potential capacity of digest and absorb nutrients. A customized PCR array was designed to study the transcriptomic response. As discussed by numerous authors (Nayak, 2010), probiotics enhance the piscine immune system. The present results confirmed these effects, as significant changes were found in the expression of key genes involved in innate and acquired immunity (il10, il12 and tlr2), that resulted upregulated in fish fed diet C as compared to the control group. The composition analysis of the gut microbiota showed a higher Firmicutes/Bacteroidetes and Proteobacteria/Firmicutes ratios in the sea bream fed diets containing probiotic compared to control fish. Despite the physiological effects on the host are difficult to assess from genomic data alone, these results could be correlated to their better growth performances (Magne et al., 2020; Rimoldi et al., 2020). Accordingly, although the microbial gut-adherent analysis reveal a lack of colonization of the probiotic in the host's intestinal mucosa, the functional prediction showed that the microbiota of fish that received probiotic was more involved in the digestion and absorption of protein. This confirms that positive modulation of fish gut microbiota can occur without probiotics colonization in the intestinal environment.

Conclusion

Our results highlight the interactions between diet and fish microbiota, suggesting that manipulating fish microbiome, through the use of well-designed probiotics, may represent a promising intervention to improve fish growth performances and digestive capacity.

(Continued on next page)

Bibliography

Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Gironés, O., and Múzquiz, J. L. (2007). In vitro competitive adhesion and production of antagonistic compounds by lactic acid bacteria against fish pathogens. *Vet. Microbiol*. 122, 373–380. doi:10.1016/j.vetmic.2007.01.023.

Borch, K., Pederson, I. E., and Hogmo, R. O. (2015). The use of probiotics in fish feed for intensive aquaculture to promote healthy guts. *Adv. Aquac. Fish. Manag*. 3, 264–273.

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., et al. (2020). The firmicutes/bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients* 12. doi:10.3390/nu12051474.

Martínez Cruz, P., Ibáñez, A. L., Monroy Hermosillo, O. A., and Ramírez Saad, H. C. (2012). Use of Probiotics in Aquaculture. *ISRN Microbiol*. 2012. doi:10.5402/2012/916845.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., et al. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302, 1–18. doi:10.1016/j.aquaculture.2010.02.007.

Nayak, S. K. (2010). Probiotics and immunity: A fish perspective. Fish Shellfish Immunol. 29, 2–14. doi:10.1016/j.fsi.2010.02.017.

Newaj-Fyzul, A., Al-Harbi, A. H., and Austin, B. (2014). Review: Developments in the use of probiotics for disease control in aquaculture. *Aquaculture* 431, 1–11. Available at: http://dx.doi.org/10.1016/j.aquaculture.2013.08.026.

Ringø, E., Zhou, Z., Vecino, J. L. G., Wadsworth, S., Romero, J., Krogdahl, Å., et al. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquac. Nutr.* 22, 219–282. doi:10.1111/anu.12346.

Rimoldi, S., Gini, E., Koch, J. F. A., Iannini, F., Brambilla, F., and Terova, G. (2020). Effects of hydrolyzed fish protein and autolyzed yeast as substitutes of fishmeal in the gilthead sea bream (*Sparus aurata*) diet, on fish intestinal microbiome. *BMC Vet. Res*. 16:118, 1–13. doi:https://doi.org/10.1186/s12917-020-02335-1.