



Article

Metagenomics Analysis Reveals an Extraordinary Inner Bacterial Diversity in Anisakids (Nematoda: Anisakidae) L3 Larvae

Susana C. Arcos ¹, Felipe Lira ², Lee Robertson ^{1,3}, María Rosa González ¹, Noelia Carballada-Sangiao ⁴, Isabel Sánchez-Alonso ⁵, Laura Zamorano ⁶, Mercedes Careche ⁵, Yolanda Jiménez-Ruiz ¹, Ricardo Ramos ⁷, Carlos Llorens ⁸, Miguel González-Muñoz ⁴, Antonio Oliver ⁶, José L. Martínez ² and Alfonso Navas ^{1,*}

- ¹ Museo Nacional de Ciencias Naturales, Dpto Biodiversidad y Biología Evolutiva, CSIC, 28006 Madrid, Spain; scobacho@mncn.csic.es (S.C.A.); robertson.lee@inia.es (L.R.); mrosaglopez@mncn.csic.es (M.R.G.); yjr@mncn.csic.es (Y.J.-R.)
 - ² Centro Nacional de Biotecnología, Departamento de Biotecnología Microbiana, CSIC, 28049 Madrid, Spain; felipelira3@gmail.com (F.L.); jlmtnez@cnb.csic.es (J.L.M.)
 - ³ Departamento de Protección Vegetal, INIA, 28040 Madrid, Spain
 - ⁴ Servicio de Inmunología, Hospital Universitario La Paz, 28046 Madrid, Spain; noeliacarba@hotmail.com (N.C.-S.); mgonzalez_munoz@hotmail.com (M.G.-M.)
 - ⁵ Instituto de Ciencia y Tecnología de Alimentos y Nutrición, CSIC, 28040 Madrid, Spain; isabel.sanchez@csic.es (I.S.-A.); mcareche@ictan.csic.es (M.C.)
 - ⁶ Servicio de Microbiología y Unidad de Investigación, Hospital Son Espases, (IdISPa), 07120 Palma de Mallorca, Spain; laura.zamorano@ssib.es (L.Z.); aoliverp@yahoo.es (A.O.)
 - ⁷ Unidad de Genómica, “Scientific Park of Madrid”, Campus de Cantoblanco, 28049 Madrid, Spain; ricardo.ramos@fpcm.es
 - ⁸ Biotechvana, “Scientific Park”, University of Valencia, 46980 Valencia, Spain; carlos.llorens@biotechvana.com
- * Correspondence: anavas@mncn.csic.es

Citation: Arcos, S.C.; Lira, F.; Robertson, L.; Gonzalez, M.R.; Carballada-Sangiao, N.; Sánchez-Alonso, I.; Zamorano, L.; Careche, M.; Jiménez-Ruiz, Y.; Ramos, R.; et al. Metagenomics Analysis Reveals an Extraordinary Inner Bacterial Diversity in Anisakids (Nematoda: Anisakidae) L3 larvae. *2021*, *9*, x. <https://doi.org/10.3390/xxxxx>

Academic editor: María Teresa Gómez-Muñoz

Received: 29 March 2021

Accepted: 10 May 2021

Published: date

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Abstract: L3 larvae of anisakid nematodes are an important problem for the fisheries industry and pose a potential risk for human health by acting as infectious agents causing allergies and as potential vectors of pathogens and microorganisms. In spite of the close bacteria–nematode relationship very little is known of the anisakids microbiota. Fresh fish could be contaminated by bacteria vectored in the cuticle or in the intestine of anisakids when the L3 larvae migrate through the muscles. As a consequence, the bacterial inoculum will be spread, with potential effects on the quality of the fish, and possible clinical effects cannot be discarded. A total of 2,689,113 16S rRNA gene sequences from a total of 113 L3 individuals obtained from fish captured along the FAO 27 fishing area were studied. Bacteria were taxonomically characterized through 1803 representative operational taxonomic units (OTUs) sequences. Fourteen phyla, 31 classes, 52 orders, 129 families and 187 genera were unambiguously identified. We have found as part of microbiome an average of 123 OTUs per L3 individual. Diversity indices (Shannon and Simpson) indicate an extraordinary diversity of bacteria at an OTU level. There are clusters of anisakids individuals (samples) defined by the associated bacteria which, however, are not significantly related to fish hosts or anisakid taxa. This suggests that association or relationship among bacteria in anisakids, exists without the influence of fishes or nematodes. The lack of relationships with hosts of anisakids taxa has to be expressed by the association among bacterial OTUs or other taxonomical levels which range from OTUs to the phylum level. There are significant biological structural associations of microbiota in anisakid nematodes which manifest in clusters of bacteria ranging from phylum to genus level, which could also be an indicator of fish contamination or the geographic zone of fish capture. Actinobacteria, Aquificae, Firmicutes, and Proteobacteria are the phyla whose abundance value discriminate for defining such structures.