

Tissue-specific proteome of *Anisakis simplex* L4 larvae reveal potential molecular mechanisms involved in parasite development and pathogenicity

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Anisakis simplex one of the most prevalent parasitic nematodes (Nematoda) of marine organisms is characterized by a complex life cycle. Humans can be accidental hosts for this parasitic species. The consumption of raw or undercooked fish containing larvae may pose a serious health risk because the parasites are able to penetrate mucous membranes of the gastrointestinal tract and cause damage to the gastric and intestinal walls as well as induce allergic reactions. Therefore, *A. simplex* was acknowledged as biohazardous organism. The pathological changes caused by *A. simplex* are known as anisakiasis. Parasitic nematodes may cause severe abdominal pain, nausea and vomiting. Anisakis larvae and their secretions interfere with host immune system and induces a Th2-type immune response.

The finding that protein composition of cuticle – the outer layer of the nematodes' body – changes in different stages of development was the breakthrough discovery in parasite research. Studies into nematode surface proteins are required to deepen our understanding of parasite-host interactions and the parasite's ability to survive in a hostile environment. Another element that is important in parasite growth and development is the digestive system. In *A. simplex*, the intestine lumen of L3 larvae is shrunken, and intestinal patency is restored in L4 larvae. It is believed that in developing organisms, it "takes over" selected nutritional functions from the cuticle. Recent reports indicate that in humans *A. simplex* larvae can develop to L4 stage. There is a lack of knowledge about the functions of the cuticle of the L4 development stage, as well as the molecular mechanism of digestion in L4 A. simplex intestine is poorly described.

Although several approaches have been employed to study the biology of nematodes and their interactions with the host, the proteomics of those two particular tissues: cuticle and intestine in the L4 stage has not been studied before. All this prompted us to analyze proteomic profiles of intestinal and cuticle cells of the L4 *A. simplex* development stage and identify potential proteins involved in parasite development and pathogenicity. To meet this goal *A. simplex* L4 larvae was used as a research model. Total protein was extracted from the intestine and cuticle tissues. Subsequently, using TMT- based (tandem mass tags) quantitative proteomics the proteomes of parasite intestine and cuticle cells were analyzed. The analysis was divided into four stages: (1) trypsin digestion assisted with high intensity focused ultrasound (HIFU), (2) TMT-isobaric

mass tag labeling, (3) proteome analysis (LC-MS/MS) of parasite's tissues using an LTQ-Orbitrap Elite mass spectrometer, and (4) identification of potential proteins involved in parasite development and pathogenicity.

Gene ontology (GO) term was performed by PANTHER classification system to understand molecular function and biological processes of the identified proteins. Then, KEGG pathway analysis by DAVID 6.8 was used to ascribe identified proteins to particular biological mechanisms and cellular pathways. The interaction network analysis between identified proteins was done with use of the STRING v10.0 software.

The tissue-specific proteomic analysis of L4 *A. simplex* stage has not yet been profoundly studied. The obtained results should provide a better understanding of molecular processes underlying the development of A. simplex infection in humans and will expand the existing knowledge about the role of cuticle in host-parasite communication. This knowledge, in turn, will allow for finding effective treatments to cure the disease. It is also possible that the presented results will help to develop future studies leading to *A. simplex* eradication.

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