

*Anisakis simplex* is a parasitic nematode that colonizes marine organisms around the world. Massive infestation by nematode larvae has been observed in Baltic herring and cod. Larvae were detected in 80% of harvested fish. The consumption of raw or inadequately prepared fish is the main source of nematode infections in humans. With progress in globalization, the popularity of exotic foods and dishes such as Japanese sushi and sashimi, Hawaiian lomi-lomi (raw salmon), South American cebiche, Spanish boquerones vinagre, Nordic gravlax (dried, smoked salmon), Dutch salted and smoked herrings, and other marinated or cold-smoked fish, continues to increase in Poland. New culinary trends involving the consumption of raw fish increase the geographic range of parasitic nematodes and the incidence of anisakiasis. Anisakiasis is a relatively new and rapidly spreading zoonotic disease that poses a serious threat for humans and animals. The disease causes economic losses in the fishing sector because it undermines consumer confidence and lowers the demand for potentially infected fish.

The consumption of fish containing invasive L3 larvae of *A. simplex* may pose a serious health risk because these parasites are able to penetrate mucous membranes of the gastrointestinal tract and induce allergic reactions in humans. Invasive larvae are resistant to freezing, cooking, marinating and salting, which makes them difficult to eliminate. The resistance of parasitic larvae continues to elude scientists who are attempting to develop effective methods for diagnosing and treating anisakiasis and neutralizing invasive larvae.

In *A. simplex*, the colonic lumen of L3 larvae is shrunken, and intestinal patency is restored in L4 larvae after the third molting. It is believed that in developing organisms, it "takes over" selected nutritional functions from the cuticle - the outer layer of the nematode's body.

A comparison of metabolic processes in L3 and L4 larvae, with special emphasis on tissues responsible for nutrition (intestine) and protection against the external environment (cuticle), can expand our understanding of the biological mechanisms that condition larval survival. Most developmental stages of parasitic nematodes take place under anaerobic conditions, and larvae derive most of their energy from saccharides. Glucose absorption from the intestinal lumen has not been described in *A. simplex* to date, and little is known about the transport of glucose across the cuticle in L3 larvae. The results of our preliminary research revealed the presence of mRNA of two facilitated glucose transporters (FGT) in L3 larvae from the Baltic population of *A. simplex*. The presence of FGT proteins was also found in L3 and L4 larvae from the Atlantic population of *A. simplex*. Differences between L3 and L4 larvae were observed.

The results of our preliminary research and literature data have prompted us to formulate the following research hypotheses: 1) L3 and L4 larvae of *A. simplex* nematodes from Baltic and Atlantic populations differ in transcriptome and proteome profiles at the global (whole larvae) and tissue (intestine and cuticle) level; 2) the transporters responsible for delivering saccharides to nematode cells are present in intestinal and cuticular cells of L3 and L4 larvae of *A. simplex*. The following research objectives have been formulated to verify the above hypotheses: 1) to identify the differences and similarities in the transcriptome and proteome profiles of L3 and L4 larvae from the Baltic and Atlantic populations of *A. simplex* in whole larvae and in selected tissues; 2) to create a data repository in the form of a pool of transcripts and proteins of whole larvae and tissues of *A. simplex* as a model organism for studies investigating the physiological and biochemical parasitic adaptations to the host and the immunopathology of parasitic diseases; 3) to select key proteins for the growth and development of parasitic nematodes; 4) to determine the type and mechanism of saccharide transport from the external environment to *A. simplex* larvae from the Baltic population.

The experimental material will be *A. simplex* L3 larvae isolated from Baltic herring and cod, while nematodes from the Atlantic population will be isolated from Atlantic cod. L3 larvae will be cultured *in vitro* until they molt into L4 larvae. Molecular methods (single-cell RNA-sequencing and LC-MS/MS analysis) will be used to identify genes and proteins in whole larvae and in selected tissues (intestinal and cuticular cells). The mechanism by which glucose is transported to the nematode's body will be investigated with the use of fluorescence methods.

The proposed project will help elucidate the mechanisms which condition the survival of nematodes in definitive hosts (marine mammals) and accidental hosts (humans). An analysis of differences in transcriptome and proteome profiles and the identification of key proteins that determine the survival of invasive L3 larvae and L4 larvae will provide valuable information in fight against anisakiasis and for designing new studies investigating parasitic infections in humans and animals based on *A. simplex* organism.