

Effect of temperature and starvation on the metabolism of trehalose in the third larval stage (L₃) of *Anisakis simplex* (Nematoda)

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Anisakis simplex is a parasitic gastrointestinal nematode with a complex life cycle; the definitive hosts — marine mammals — as well as in humans. As anisakiosis is a serious condition that be fatal to a patient and may cause allergic reaction in patients sensitive to *A. simplex* allergens, it is necessary to intensify research on biochemistry of the parasite larvae. For example, little is known about its carbohydrates metabolism, in particular — trehalose. This sugar is of special importance for parasites owing to its physical and chemical properties. Besides the function of energy reserve, it fulfills a protective role under stress conditions. Free-living and parasitic nematodes synthesize trehalose in reaction to desiccation and cooling. In parasites of animals trehalose is the reserve sugar and a transport from supplying glucose to tissues.

There is no information available on synthesis of trehalose in the third larval stage of *A. simplex* that's why in the present research we decided to mark determine the activity of enzymes participating in synthesis and decomposition of trehalose and content of trehalose during starvation of *A. simplex*. Activity of TPS (EC 2.4.1.15), was determined using the method by Giaever et al. (1988), and that of TPP (EC3.1.3.12) by Kaasen et al. (1992). The end of the product of reaction — trehalose and content of that sugar was determined using HPLC. Trehalase (EC 3.2.1.28) activity was measured using a modified Dahlqvist method (1968), protein content according to Bradford (1976).

The presence of activity of TPS (21.013 ± 0.034 u/mg) and TPP (19.367 ± 0.041 u/mg) in L₃ of *A. simplex* is shown for first time in this paper. Experiment of culturing L₃ larvae without nutrients at five temperature, i.e. 0, 4, 10, 37 and 45°C was carried out. During the 6 hours of kipping the larvae at 4, 10 and 45°C the content of trehalose increased by 70% than that measured in the larvae freshly isolated from fish. In this time activity of trehalase was the same at the beginning cultivation. Activity of enzyme of synthesis of trehalose decreasing rapidly during all the time experiment. During the following hours of starvation at 0, 4 and 45°C the content of trehalose was maintained at a similar level and on 18h and 30h it was lower by ca 70% than at the beginning of the experiment.

These results demonstrate that enzymes responsible for both pathways of trehalose metabolism: anabolism and catabolism are present in *A. simplex*.