Diversity and activity of sugar transporting genes of *Anisakis simplex* s. s. L3 and L4 larvae, *in vitro* analysis

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Background

The larvae of genus Anisakis nematodes responsible for disease known as anisakiasis in humans. *Anisakis simplex* is as a model organism due to the possibility of *in vitro* culture of both larval L3 and L4, different functions of the digestive system an additional advantage is the fact that only these stages have been identified as a pathogenic factor in humans.

Currently very little is known of the process by which parasite nematodes take up glucose. In the present study we address this problem by characterizing the GLUT-like transcripts in *A. simplex* and by investigating the relationship of their proteins with glucose.

It is hypothesized that *A. simplex* uses glucose transporters as like a nematode species, *Caenorhabditis elegans*.

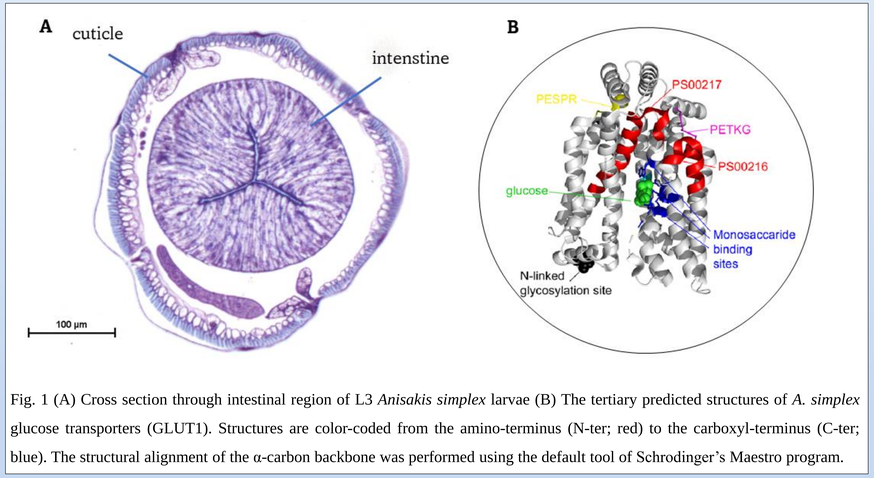

Fig. 1 (A) Cross section through intestinal region of L3 *Anisakis simplex* larvae (B) The tertiary predicted structures of *A. simplex* glucose transporters (GLUT1). Structures are color-coded from the amino-terminus (N-ter; red) to the carboxyl-terminus (C-ter; blue). The structural alignment of the α-carbon backbone was performed using the default tool of Schrodinger's Maestro program.

Material and Methods

L3 and L4 larvae of *A. simplex* s.s. was cultured *in vitro* according to Iglesias et al. (2001) using medium RPMI-1640 with glucose at concentration of range from 0.1 to 20 mg/mL for 12h. The larvae from each sample were used for total RNA isolation. The gene expression levels (mRNA) of five facilitated glucose transporters (FGT 1, FGT 2, FGT 3, FGT 5, FGT 9) and sugar transporter (SWEET 1) was determited by the Real-time PCR. Gene expression profiles were calculated using the comparative Pfaffl method (2001).

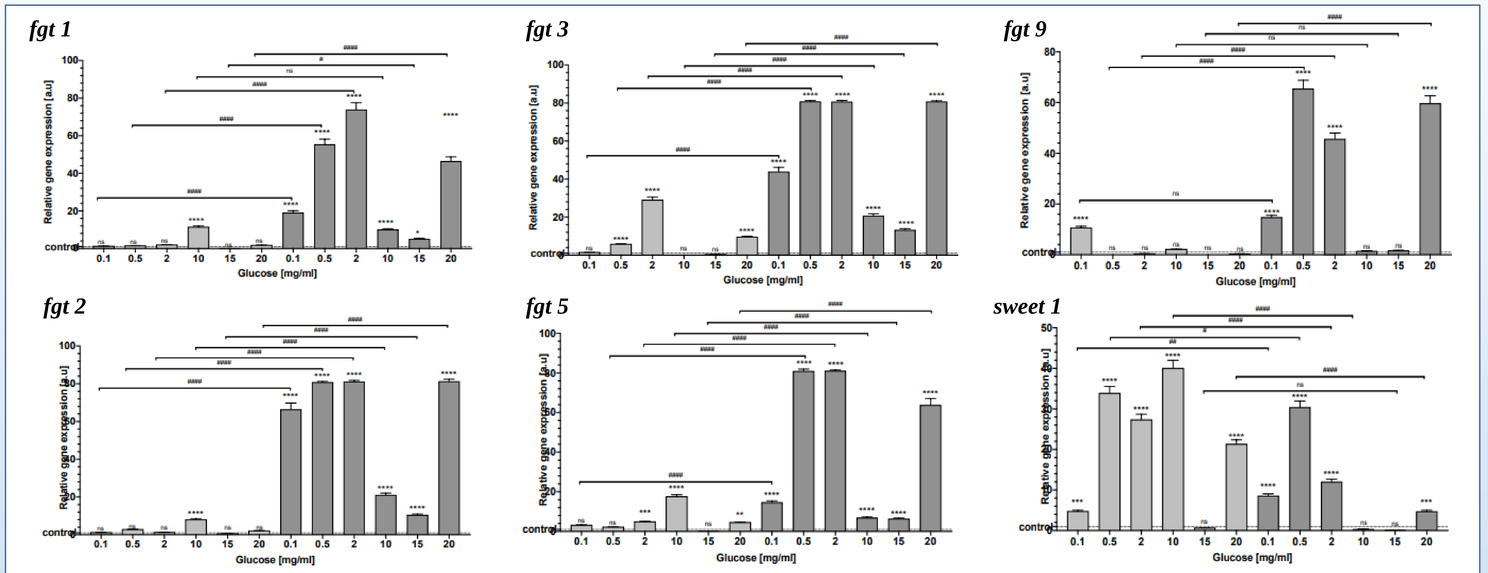

Fig. 2 *Anisakis simplex* glucose transporters genes expression after *in vitro* incubation with glucose for 12h

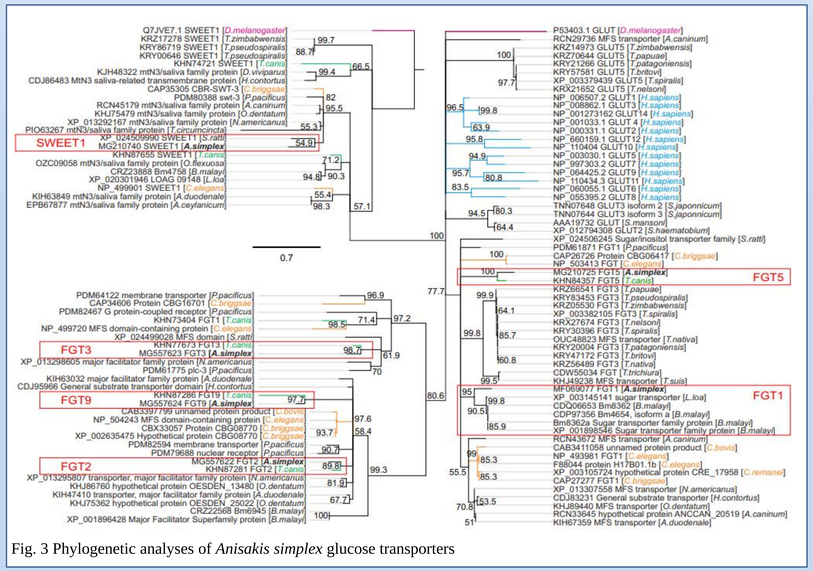

Fig. 3 Phylogenetic analyses of *Anisakis simplex* glucose transporters

Results

We obtained full-length sequences of 5 putative GLUT (glucose transporter)-like genes (FGT1, FGT2, FGT3, FGT5, FGT9) and SWEET1 from *A. simplex*. After 12 hours treatment only SWEET1 was demonstrated expression in both larval stages. The mRNA expression of glucose transporters in L3 larvae after 12 hours of sugar stimulation indicate a lack of response to an external stimulus as soon as only in the L4 larvae a positive response was recorded [Fig. 2]. Concurring with the transcription profiles, our phylogenetic analyses revealed that *A. simplex* glucose transporters belong to two separate clusters, one associated with class I glucose transporters from *C. elegans*, and the other specific to parasitic clad III of Nematode [Fig. 3].

Conclusions

Analysis of the expression glucose transporters of *A. simplex*, as a representative of parasitic nematodes, will affect the better understanding of the biology of parasitic nematodes and will allow finding ways to overcome the diseases caused by them.

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