

Prace oryginalne

Hydrolases of *Hysterothylacium aduncum* (Nematoda)*

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ABSTRACT. **Background**. Enzymatic activity is an indicator of an organism's metabolic rate which depends on, i.e., environmental conditions, developmental stage, physiological stage, and sex. The API ZYM test was applied to compare activities of 19 hydrolases of female and male *Hysterothylacium aduncum*. **Material and methods**. Sexually mature nematodes were isolated from eelpout individuals caught in the Gulf of Gdańsk. Enzymatic activity of the hydrolases and the protein content was determined in nematode extracts using API ZYM and Bradford's method, respectively. **Results**. The females and males tested showed a total of 13 enzymes to be active. The males showed additionally the presence of α-fucosidase. Acidic and alkaline phosphatases had very high activities in both sexes; short-chain fatty acid esterases, leucine and valine aminopeptidases, α-glucosidase, and N-acetylglucosaminidase were highly active. *H. aduncum* showed no trypsin- and chymotrypsin-specific activities; similarly, no activity of α-galactosidase, α-mannosidase, and ß-glucuronidase was revealed. Except for lipase (C14), hydrolases were more active in females than in males, which is related to metabolic rate being higher in females due to their reproductive function. **Conclusion**. Comparison of the results obtained with earlier data produced with API ZYM allowed suggesting that the hydrolase pattern may be more affected by habitat in the host than by the taxonomic affiliation of nematode.

Key words: Anisakidae, API ZYM, hydrolases, *Hysterothylacium aduncum*.

Introduction

Hysterothylacium aduncum is a highly prevalent, cosmopolitan nematode, parasitic in fish alimentary tract [1–3]. The parasite occurs in the Baltic fish [4, 5]. Recently, Rokicki [6] reported on the parasite's ability to complete its life cycle in the Vistula Lagoon. While the morphology, life cycle, and taxonomy of *H. aduncum* are fairly well known [5–9], data on the parasite's biochemistry and physiology are very scant [10-12]. Our earlier study [11] addressed carbohydrate contents and activity of enzymes responsible for catabolism of glycogen and trehalose in *H. aduncum*. We were able to demonstrate the presence of enzymes involved in phosphorolytic and hydrolytic pathways of glycogen and trehalose breakdown both in the larvae and in the adult nematodes. This work was aimed at finding out whether extracts of mature males and females of *H. aduncum* also contain enzymes hydrolysing substrates other than carbohydrates, and — if such enzymes do occur — what their activity is. We selected API ZYM as a method of choice; the test is capable of simultaneous measurement of the activities of as many as 19 hydrolases of different specificity, targeting 3 major biomolecules: proteins, lipids, and carbohydrates [13]. Knowledge about the activity of these enzymes at *H. aduncum* will let pointing out the group of the most essential energy substrates for this species, and suggest what major metabolites are appearing during life processes of this parasite. The informations about metabolism of *H. aduncum* may be important for the control of this parasite.

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Material and methods

The nematodes were isolated from eelpout individuals caught in December 2005 in the Gulf of Gdańsk. After rinsing in 0.6% NaCl, sexually mature females and males were picked out. They were weighed and homogenized, in a glass Potter vessel, with three volumes of 0.6% NaCl. The homogenate was centrifuged at 1500 *x* g in a refrigerated centrifuge. The supernatant was assayed for protein content, using Bradford's method [14]. The supernatant was diluted to 2 mg protein/ml. Subsequently, 50 µl portions of the extract were applied to API ZYM (bioMérieux Lyon, France) and the test was performed as instructed by the manufacturer. The results were expressed as scores of the 5-score enzyme activity scale, where: 0 = no activity; 1 = 5 nmol/mg protein; 2 = 10 nmol/mg; 3 = 20 nmol/mg; 4 = 30 nmol/mg; and 5 = 40 nmol/mg [15]. The results reported are means of 5 assays.

Results

Data on hydrolase activities in the female and male *H. aduncum* are summarised in Table 1. API ZYM detected 13 and 14 hydrolases (out of 19 possible) in the females and males, respectively. The enzymes were identical in both sexes, except for α-fucosidase not detected in the females.

Extracts of *H. aduncum* showed all the esterases tested to be active (Table 1). Particularly high was the activity of phosphatases. The enzymes were somewhat more active in the females than in the males. Esterases active upon short-chain fatty acid esters were, too, slightly more active in the females. On the other hand, lipase (C14) hydrolysing lipids in the form of long-chain fatty acid esters was twice more active in the *H. aduncum* males than in the females.

Enzymes breaking down proteins and peptides were represented only by aminopeptidases (arylamidases in Table 1). The highest activity in both sexes was typical of leucine aminopeptidase, followed by valine aminopeptidase and cysteine aminopeptidase. Activities of the latter two enzymes in the females were twice of those in the males. No trypsin- and chymotrypsin-specific activity was detected (Table 1).

Of 8 glycosidases tested, activities of 3 (α-galactosidase, α-mannosidase, and β-glucuronidase) were not detected (Table 1). Activity of β-glucosidase was very low. The highest activity was typical of N-acetyl-β–glucosaminidase. Glycosidase activities were usually higher in the females than in the males. For example, activity of α-glucosidase in the females was as much as twice that in the males. The males tested positively for the presence of α-fucosidase the activity of which, as already mentioned, was not detected in the female *H. aduncum* (Table 1).

Discussion

The metabolic activity of an organism changes during ontogenesis and depends on its physiological state which is in turn affected by internal controls and external factors.

The API ZYM test is a good tool enabling observation of changes in the activity of enzymes during the development of parasites [16]. It may be also used to compare profiles of hydrolases present in representatives of various taxa [13, 17]. Application of API ZYM allowed performing a comparative analysis of hydrolases present in nematodes in which the enzymes had been assayed [16–19]. Phosphatases of all the parasites subjected to API ZYM were found to be highly active. On the other hand, the parasites differed in their activities of esterases acting upon fatty acid esters, peptidases, and proteinases as well as glycosidases. The *H. aduncum* hydrolase pattern found in this work was similar to that revealed, with the same test, in other nematodes parasitic in vertebrates [16, 18, 19]. The pattern was, however, markedly different from that demonstrated for entomopathogenic nematodes, parasitoids of insects [17] which showed the presence and high activity of all enzymes belonging to the subclass of glycosidases. Active were also trypsin and chymotrypsin, the activities of which were not detected in the nematodes parasitic in fish: *H. aduncum* (Table 1), *A. simplex* and *C. farionis* [16, 18]. On the other hand, Dziekońska-Rynko et al. [19] demonstrated chymotrypsin with low activity in *Contracaecum rudolphi*. The negative result of the test for protease activity in nematodes parasitic in fish may be explained by differences in their substrate specificity. As reported by Łopieńska-Biernat et al. [16] and Dziekońska-Rynko et al. [18], the larval *A. simplex* as well as larvae and adult *C. farionis* showed protein-digesting proteases to be active in alkaline and acidic media with no simultaneous activity of trypsin and chymotrypsin against substrates specific

Table 1. Activity of hydrolases in extracts from male and female *Hysterothylacium aduncum*

No.	Enzyme	Substrate	pH	Activity (nmol/mg) male	female
					Esterases
1	Alkaline phosphatase	2-naphtyl phosphate	8.5	35	40
2	Acid phosphatase	2-naphtyl phosphate	5.4	30	40
3	Naphtol-AS-BI-phosphohydrolase	Naphtol-AS-BI-phosphate	5.4	37.5	40
4	Esterase (C4)	2-naphtyl butyrate	6.5	30	32.5
5	Esterase lipase (C8)	2-naphtyl caprylate	7.5	20	25
6	Lipase (C14)	2-naphtyl myristate	7.5	20	10
					Peptidases and Proteases
7	Leucine arylamidase	L-leucyl-2-naphtylamide	7.5	40	40
8	Valine arylamidase	L-valyl-2-naphtylamide	7.5	15	30
9	Cystine arylamidase	L-cystyl-2-naphtylamide	7.5	10	20
10	Trypsin	N-benzoyl-DL-arginine-2-naphtylamide	8.5	0	0
11	Chymotrypsin	N-glutaryl-phenylalanine-2-naphtylamide	7.5	0	0
					Glycosidases
12	α-galactosidase	6-Br-2-naphtyl-α-D-galactopyranoside	5.4	0	0
13	ß-galactosidase	2-naphtyl-ß-D-galactopiranoside	5.4	20	25
14	ß-glucuronidase	Naphtol-AS-BI-ßD-glucuronide	5.4	0	0
15	α-glucosidase	2-naphtyl-α-D-glucopiranoside	5.4	15	30
16	ß-glucosidase	6-Br-naphtyl-ß-D-glucopyranoside	5.4	5	5
17	N-acetyl-ß-glucosaminidase	1-naphtyl-N-acetyl-ß-D-glucosaminide	5.4	35	40
18	α-mannosidase	6-Br-2-naphtyl–α-D-mannopyranoside	5.4	0	0
19	α-fucosidase	2-naphtyl-α-L-fucopyranoside	5.4	20	0

of those enzymes used in API ZYM (see Table 1).

It is interesting to compare activities of hydrolases, other than proteases, of three parasitic nematodes belonging to the Anisakidae: *A. simplex*, *H. aduncum*, and *C. rudolphi*. Activity of glycosidases in extracts of adult *C. rudolphi*, nematodes occurring in the stomach and intestine of piscivorous fish, was usually higher; moreover, the extracts contained all the enzymes tested, belonging to that subclass of hydrolases, except α-galactosidase [19]. Larvae of *A. simplex* [16] and *H. aduncum* (Table 1), occurring in the fish body cavity and digestive tract, showed no activity of two other glycosidases in addition to α-galactosidase. Among the glycosidases tested, α-fucosidase in the L_3 larvae of *A. simplex* and in the male *H. aduncum* was highly active. This enzyme can selectively cleave fucose-containing glycoproteins and glycoconjugates [20]. In our opinion, the enzyme is important for the larval *A. simplex* during tissue penetration. Since adult *H. aduncum* are not parasites penetrating tissues of the host their female have not α-fucosidase, but it is possible to connect the presence of this enzyme at males with its participation in the process of fertilization, when glycoproteins of eggs membranes are being degraded [21].

Sexual dimorphism in dioecious nematodes is reflected also at the biochemical level [22], which is confirmed by the results obtained in this study. With the exception of lipase, hydrolases activities in the females were higher (sometimes much higher) than in the males (Table 1). This is understandable in view of higher metabolic needs of mature females, related to their production of numerous eggs which contain storage materials. Valine and cysteine aminopeptidases were twice as active in the female *H. aduncum* as in the males (Table 1). Among the carbohydrate catabolism enzymes, female α-glu-

cosidases were twice as active as those of the males, which is in agreement with our earlier observations [11]. Using conventional enzymatic tests, we were able to demonstrate very high activities of trehalase, maltase, and glucoamylase in adult *H. aduncum*, the enzymes belonging to the same subclass of hydrolases. In addition, concentration of glucose, the major product of α-glucosidase activity against saccharides, was 10 times higher in the adults than in the larval stages [11].

To conclude, although the female and male hydrolase patterns were identical, the hydrolase activity level was sex-dependent. The only exception was provided by α-fucosidase, an enzyme which occurs only in males. Comparison of results obtained in this work with earlier data produced by the same test allows us to suggest that a parasite's hydrolase pattern depends more on the type of host's habitat than on the parasite's taxonomic affiliation. This was confirmed by the observation that the pattern and activity of hydrolases of adult *H. aduncum*, determined in this study, as well as of *A. simplex* L_3 larvae [16] were more similar to *C. farionis* [18], a nematode parasitizing fishes and belonging to a different family, that to *C. rudolphi*, a member of the family Anisakidae, the adults of which were isolated from cormorants [19].

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