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THE ACTIVITY OF HYDROLASES OF ENTOMOPATHOGENIC NEMATODES

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ABSTRACT. Similar patterns of hydrolases were observed in three species representing two genera of entomopathogenic nematodes: *Steinernema affinis*, *S. feltiae*, and *Heterorhabditis zealandica*. The same enzymes were present in the studied nematodes but they differed in the level of activity of individual sub-classes of enzymes. A higher activity of esterases and proteolytic enzymes could be observed for *H. zealandica* than for *S. affinis* and *S. feltiae*. On the other hand, the activity of glycosidases in steinernematids was generally much higher than in *H. zealandica*.

Key words: entomopathogenic nematodes, *Heterorhabditis zealandica*, hydrolases, *Steinernema affinis*, *S. feltiae*.

Entomopathogenic soil-living nematodes of families Steinernematidae and Heterorhabditidae are mutualistically associated with bacteria of genus *Xenorhabdus* and *Photorhabdus* spp. After penetrating into the insect through the natural openings or through the body walls the infective juveniles of the nematodes release the symbionts. The bacteria multiply in the haemolymph of the insect causing septicaemia resulting in death of the host within 48 h (Poinar 1990). Entomopathogenic nematodes are increasingly frequently used for biological control of crop pests (Smart 1995). The loss of infectivity of nematodes, progressing with time, is the major challenge (Friedman 1990). That problem also appears in case of extended storage of nematode larvae bread for commercial purposes and in field conditions (Grewal 2000).

As the free-living forms of nematodes do not feed, their condition is linked to the volume and type of accumulated reserve compounds. Lipids and glycogen are their major energetic substrates (Patel and Wright 1997a, b; Qiu et al. 2000). The rate and sequence of utilization of them vary and depend on the nematode species (Patel et al. 1997, Wright et al. 1997, Fitters et al. 1999, Qiu et al. 2000).

Adaptability of nematodes to extreme temperatures was studied extensively as well as changes in the composition of fatty acids in lipids (Hatab and Gaugler 1997). Variable expression of isoenzymes and effect of the temperature on the activity of glucose-6-phosphate dehydrogenase and hexokinase, the major enzymes of the pentose cycle, and glycolysis in steinernematids following laboratory recycling

were investigated (Jagdale and Gordon 1997, 1998). Shih et al. (1996) characterized the key enzymes of glycolysis and Krebs cycle in *Steinernema carpocapsae*. No information concerning hydrolases important for utilization of reserve materials as well as those necessary in the processes of host penetration by infective larvae of entomopathogenic nematodes has been found so far.

In this study it was decided to use API ZYM test to show the presence and to identify and compare the activity of hydrolases of *Steinernema affinis*, *S. feltiae* (Steinernematidae), and *Heterorhabditis zealandica* (Heterorhabditidae).

MATERIAL AND METHODS

Polish populations of *S. affinis, S. feltiae*, and *H. zealandica* were received from Dr. Elżbieta Pezowicz from the Division of Zoology, SGGW in Warsaw. All nematodes were propagated through wax moth larvae (*Galleria mellonella*). The infective juveniles (IJs) were harvested 14 days post-infection and stored in aerated 0.01% formalin at 4°C.

Extraction of enzymes was performed according to Jagdale and Gordon (1997). The IJs were homogenized with 0.9% NaCl. The homogenate was centrifuged at 2500 g for 15 min. Protein in supernatant was assayed according to Bradford's method (1976). 50 µl of enzymatic extract containing 100 µg of protein was placed on API ZYM test cupule (bioMérieux Lyon, France). The further procedure was consistent with manufacturer's instructions. The test allowed identification of as many as 19 different hydrolases using a relatively small volume of the enzymatic extract (Table 1). The activity of enzymes was determined according to 5-point scale where 0 represented lack of activity, 1 = 5 nmol/mg^{-1}, 2 = 10 nmol/mg^{-1}, 3 = 20 nmol/mg^{-1}, 4 = 30 nmol/mg^{-1} and 5 the maximum activity > 40 nmol/mg^{-1} (Głowacka and Ochęcka-Szymańska 2001). The results presented are the mean of four repetitions for larvae of each species.

RESULTS AND DISCUSSION

The results obtained (Table 1) showed the same pattern of hydrolases. The same enzymes were present in the studied nematodes of Steinernematidae and Heterorhabditidae families. However, representatives of those families differed in the level of activity of individual sub-classes of enzymes. Higher activity of esterases and proteolytic enzymes could be observed for *H. zealandica* than for steinernematids. On the other hand, the activity of glycosidases in nematodes of that later family was generally much higher than for *H. zealandica*.

Alkaline and acid phosphorylases showed very high activity in case of all studied species. Only in case of *S. feltiae* it was lower. Also the esterases that hydrolyse the esters of short fatty acids (C4 and C8) showed high activity. Absence of li-

pase acting on esters of longer fatty acids in all species of nematodes was a surprise. This is interesting as acids C16 and C18 are the major components of lipids and phospholipids in representatives of both Steinernematidae and Heterorhabditidae (Patel and Wright 1997b, Fitters et al. 1999). What is more, as indicated by those studies, they are utilized during storage of nematode larvae. It is possible that our negative results are a consequence of the fact that the nematodes used were stored no longer than 18 days after emergence from the host's body. During that time the activity of lipases may be low making its invisible under the conditions of our test. A certain confirmation to that suggestion can be found in studies by Wright et al. (1997), who, in case of *S. feltiae*, observed mainly utilization of glycogen during the initial period of storage followed by utilization of lipids.

It may be assumed after Shih et al. (1996) that pH 7.5 is physiological for the body of entomopathogenic nematodes but the activity of alkaline proteases – trypsin and chymotrypsin, was relatively low (Table 1). Impossibility of identifying proteolytic enzymes active in acid environment is the weakness of the test applied. It is possible that in their case, similarly to other parasitic nematode, the activity of acid proteases may be high (Łopieńska et al. 2000). Among peptidases, leucine arylamidase showed activity several times higher than valine- and cystine arylamidases (Table 1).

Table 1. Assayed enzymes, their substrates and activity (nmol/mg^{-1}protein)

No.	Enzyme	Substrate	pH	Activity *S. affinis*	*S. feltiae*	*H. zealandica*
		Esterases				
1	Alkaline phosphatase	2-naphtyl phosphate	8.5	>40	35	>40
2	Acid phosphatase	2-naphtyl phosphate	5.4	>40	25	>40
3	Naphtol-AS-BI -phosphohydrolase	Naphtol-AS-BI-phosphate	5.4	30	25	>40
4	Esterase (C4)	2-naphtyl butyrate	6.5	>40	27.5	>40
5	Esterase lipase (C8)	2-naphtyl caprylate	7.5	37.5	22.5	40
6	Lipase (C14)	2-naphtyl myristate	7.5	0	0	0
		Peptidases and Proteases				
7	Leucine arylamidase	L-leucyl-2-naphtylamide	7.5	>40	35	>40
8	Valine arylamidase	L-valyl-2-naphtylamide	7.5	10	17.5	20
9	Cystine arylamidase	L-cystyl-2-naphtylamide	7.5	5	10	15
10	Trypsin	N-benzoyl-DL-arginine-2-naphtylamide	8.5	7.5	10	15
11	Chymotrypsin	N-glutaryl-phenylalanine-2-naphtylamide	7.5	10	10	5
		Glycosidases				
12	α-galactosidase	6-Br-2-naphtyl-α-D-galactopyranoside	5.4	10	10	5
13	ß-galactosidase	2-naphtyl-ß-D-galactopiranoside	5.4	>40	35	15
14	ß-glucuronidase	Naphtol-AS-BI-ßD-glucuronide	5.4	>40	>40	5
15	α-glucosidase	2-naphtyl-α-D-glucopiranoside	5.4	2.5	5	2.5
16	ß-glucosidase	6-Br-naphtyl-ß-D-glucopyranoside	5.4	10	10	22.5
17	N-acetyl-ß -glucosaminidase	1-naphtyl-N-acetyl-ß-D-glucosaminide	5.4	30	20	10
18	α-mannosidase	6-Br-2-naphtyl-α-D-mannopyranoside	5.4	7.5	5	2.5
19	α-fucosidase	2-naphtyl-α-L-fucopyranoside	5.4	32.5	20	5

As earlier mentioned, activity of glycosidases was much higher in studied Steinernematidae than in Heterorhabditidae (Table 1). Generally, it can be observed that their β-glucosidases are much more active than α-glucosidases. Among that first group, β-galactosidase and β-glucuronidase were particularly active (Table 1). N-acetyl-β-glucosaminidase was slightly less active. Relatively high activity of β-glucosidase in *H. zealandica* is worth noticing. Only that glycosidase has higher activity in that nematode.

Among α-glycosidases, only α-fucosidase showed high activity in steinernematids. Under the test conditions α–galactosidase and α-mannosidase showed only low activity. Very low activity of α-glucosidase was recorded in all the studied species. It was surprising as trehalose and glycogen are the major carbohydrates of the host insects of those nematodes.

API ZYM tests carried out in this study have not shown significant differences in the pattern of enzymes simultaneously with differences in the activity levels of individual enzymes between *S. feltiae*, *S. affinis*, and *H. zealandica*. The observed differences in activity of enzymes between the nematodes' genera may represent the cumulative effect of their activity in all the organisms forming the parasitoid system – the nematode and its bacterial symbiont. Moreover, it seems that repeating that test with symbiotic bacteria alone and the nematodes without bacteria would allow finding the origin, and determining the participation, of enzymes from both sources in the total activity of hydrolases, and better understanding of their pathogenicity.

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