PERGAMON

Soil Biology & Biochemistry 32 (2000) 929–934

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Activity of glycolytic enzymes in the gut of *Hormogaster elisae* (Oligochaeta, Hormogastridae)

M.H. Garvín[a,*], C. Lattaud[b], D. Trigo[a], P. Lavelle[b]

[a]*Departamento de Biología Animal I (Zoología), Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain*
[b]*Laboratoire d'Ecologie des Sols Tropicaux, ORSTOM, 32 rue H. Varagnat, 93143 Bondy Cedex, France*

Accepted 6 December 1999

Abstract

The glycolytic enzymatic activities in the gut of the endogeic earthworm *Hormogaster elisae* from El Molar (Madrid, Spain) were studied in order to determine its digestive capacity and to assess its alimentary regime. Most endogeic earthworms have weak enzymatic complement and they usually establish mutualistic relationships with soil microflora to digest some organic compounds. Therefore, the intestinal wall tissues were cultured in vitro to assess the origin of the glycolytic enzymes found in the gut and enzymatic activities were measured in both cultured tissues and culture media. *H. elisae* had a wide but not very strong enzyme complement: since all substrates were degraded but most of them at a low rate. This species cannot produce cellulase and mannanase, so for the digestion of these substrates it probably uses the digestive enzymatic capabilities of the ingested microflora. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Earthworms: *H. elisae*: Enzymes: Mutualism: In vitro culture

1. Introduction

Soil microorganisms constitute an important food resource in the earthworm diet (Edwards and Bohlen, 1996). Some bacteria may be consumed by earthworms, and therefore decrease in number, but particular groups of microorganisms seem to be stimulated during gut transit and total microbial activity is generally enhanced in earthworm guts compared with both non-ingested soil and casts (Brown, 1995; Karsten and Drake, 1995).

Geophagous endogeic earthworms have a poor digestive enzymatic capability (Lavelle, 1983), whilst the soil microflora has the ability to degrade almost any kind of organic substrates. This fact led to postulate a mutualistic relationship between soil microflora and endogeic earthworms (Lavelle et al., 1983).

In temperate zones studies about earthworm digestive enzyme activities have been conducted only in the Lumbricidae family. These works are principally focussed on cellulase and chitinase activities (Laverack, 1963; Loquet and Vinceslas, 1987; Urbášek, 1990; Urbášek and Pizl, 1991). Furthermore, the origin of the enzymes and the respective roles of earthworms and soil microflora have not really been addressed. Parle (1963) reported that most cellulase and chitinase enzymes that occur in the intestine of earthworms were secreted by the earthworms themselves and not by the symbiotic microflora. However, studies on digestive enzymes in the gut of the tropical species *Pontoscolex corethrurus* (Zhang et al., 1993) and *Millsonia anomala* (Lattaud et al., 1997a) have shown that these species cannot secrete cellulase. The degradation of this substrate seems to be carried out by microorganisms ingested together with the soil. There are no data concerning other earthworm families, especially from Mediterranean areas. Thus, the aim of this study was to identify glycolytic activities in the gut of *H. elisae*

* Corresponding author. Tel.: +34-9139-44955; fax: +34-9139-44947.
E-mail address: mhgarvin@eucmax.sim.ucm.es (M.H. Garvín).

PII: S0038-0717(99)00222-9

and to determine whether these enzymes were produced by the worm itself or by the microorganisms ingested with the soil.

2. Materials and methods

2.1. Area description

Earthworms and soil for laboratory cultures were collected at El Molar (Madrid, Spain). *H. elisae* (Álvarez, 1977) is an endogeic oligohumic member of the Hormogastridae, endemic to the centre of the Iberian Peninsula, that constitutes a monospecific population in the study site. El Molar is in a warm dry Mediterranean climate. The soil is sandy (15.40% clay, 11.20% lime, 73.40% sand), with an almost neutral pH (6.65) and poor in organic matter (0.97% C, 0.09% N, C/N=11). The vegetation is a subnitrophilic pasture.

2.2. Laboratory analysis

The worms were kept in 2 mm sieved soil at 20% humidity (dry weight) and laboratory temperature (approximately 21°C). First, the glycolytic enzyme activities were determined in the whole gut (wall+content). Seven earthworms were dissected in ice cold distilled water and the guts were divided into two parts (anterior and posterior). The anterior part comprised the pharynx, the oesophagus, the crop and the gizzard; the posterior part was further divided into three equivalent parts (foregut, midgut and hindgut). Each section was used to prepare enzymatic solutions. Briefly, the samples were homogenized and centrifuged. After that, the supernatant was dialysed for 1 day and the resulting solution was used as enzyme resource.

The second part of the study was to determine whether the enzymes found in the gut were produced by the earthworms themselves or by the ingested microflora. Before dissection, earthworms were put overnight on cellulose wool soaked with physiological solution, then for 4 h with a fungicide (Fungizone, i.e. amphotericin B). After that, worms were dissected in sterilized conditions and small sections of the gut walls were kept in vitro in a liquid culture medium with Fungizone and antibiotics (penicillin and colimycine). After the culture period, enzyme solutions of the tissues were prepared as for the whole gut. The culture medium was directly dialysed and the resulting solution was used as enzyme source. The enzymatic activities were tested both in the tissues and in the culture medium. The methodology is fully explained in Lattaud et al. (1997a).

Twenty-one substrates were tested: eight polysaccharides (starch, laminarine, lichenin, galactomannan, pullulan, carboxymethylcellulose (CMC), mannan and cellulose), seven heterosides (α-glucoside, α-galactoside, *N*-acetylglucosamine, β-mannoside, β-glucoside, β-galactoside and β-xyloside) and five oligosaccharides (maltose, laminaribiose, cellobiose, sucrose and gentiobiose). Both heterosidase and polysaccharidase activities were determined by the Somogyi and Nelson micromethod (Nelson, 1944; Somogyi, 1945). For oligosaccharidases, the glucose-oxidase method was used (Werner et al., 1970). The protein content of the solutions was also calculated following Sedmark and Grossberg (1977).

2.3. Statistical analysis

In the whole gut, the enzyme activities for every substrate in each gut section were analysed by one way ANOVA. For in vitro cultures a two-way ANOVA was used, considering the culture time and the gut section as independent variables. Multiple comparisons among variables were analysed by the Tukey's Test.

3. Results

3.1. Specific glycolytic activities in the gut

H. elisae was able to digest all the studied substrata with different intensity, although the activity on some of them, such as sucrose, was insignificant. In the anterior part of the gut (pharynx, oesophagus, crop and gizzard), the activity on all substrata was very low. Both the highest and the lowest activities were detected in the foregut and the hindgut, respectively.

The highest degraded heteroside in the whole gut was *N*-acetylglucosamine (Fig. 1A). The activity of this enzyme was significantly higher in the foregut than in the rest of the gut ($F = 36.986$; $P < 0.01$). β-Xyloside and α-glucoside showed the smallest enzyme activities whilst on the rest of the substrates the activities were low. The only oligosaccharides to be broken up at a high rate were maltose and laminaribiose, mainly in the foregut (Fig. 1B). The enzymes for the hydrolysis of both substrates showed higher values in the foregut than in the midgut ($F = 24.486$; $P < 0.05$ and $F = 25.409$; $P < 0.01$, respectively). In the hindgut there was no activity at all on any oligosaccharide. Starch and laminarin were the most degraded polysaccharides, especially in the foregut ($F = 41.644$; $P < 0.01$ and $F = 70.029$; $P < 0.01$, respectively). Lichenin and CMC also showed important activities. The hydrolysis of CMC was again much higher in the foregut ($F = 105.241$; $P < 0.01$). Xylan, pullulan, mannan and galactomannan were lower degraded (Fig. 1C). Cellulose was weakly broken up in both the foregut and the hindgut and it was not degraded at all in the midgut.

3.2. Specific activities in tissues

Only foregut, midgut and hindgut tissues were cultured since enzyme activity was barely found in the anterior part of the gut. Results confirmed that the strongest activity was located in the foregut and the weakest in the hindgut. Enzyme activities in the intestinal wall tissues were much lower than in the whole gut (more than 100 times less in some cases).

The enzyme activities for heterosides in the tissues showed that all of them were degraded and *N*-acetylglucosaminase was again the main enzyme, especially after 3-days culture ($F = 135.634$; $P < 0.01$). The weakest activity was detected on β-xyloside, α-glucoside and β-mannoside (Table 1). All the polysaccharides except mannan and cellulose were degraded. Laminarin was again the most degraded substrate, mainly after 3 days, when this enzyme reached the 50% of the total activity in the gut walls. Hydrolysis of starch gradually increased during the experimental period, so after 7 days values were significantly higher than after 3 and 5-days culture ($F = 20.628$; $P < 0.05$), reaching similar results than for laminarine. Lichenine and CMC also showed a high activity but CMC only appeared in the midgut after 7-days culture (Table 1). Concerning the oligosaccharidases, cultures confirmed that *H. elisae* did not possess such enzymes in its hindgut. Laminaribiose was the substratum that promoted main enzyme activity, but only in the foregut and after 5-days culture. Maltase had a weak activity; it was present during the whole culturing process in the foregut but disappeared in the midgut in 5 and 7-day cultures. Cellobiase had the weakest activity and appeared after 5 days (Table 1).

3.3. Total activity in the culture medium

The culture medium was full of exogenous proteins, so the results were expressed as total activity in μg reducing sugars min^{-1} (Table 2). Within heterosides,

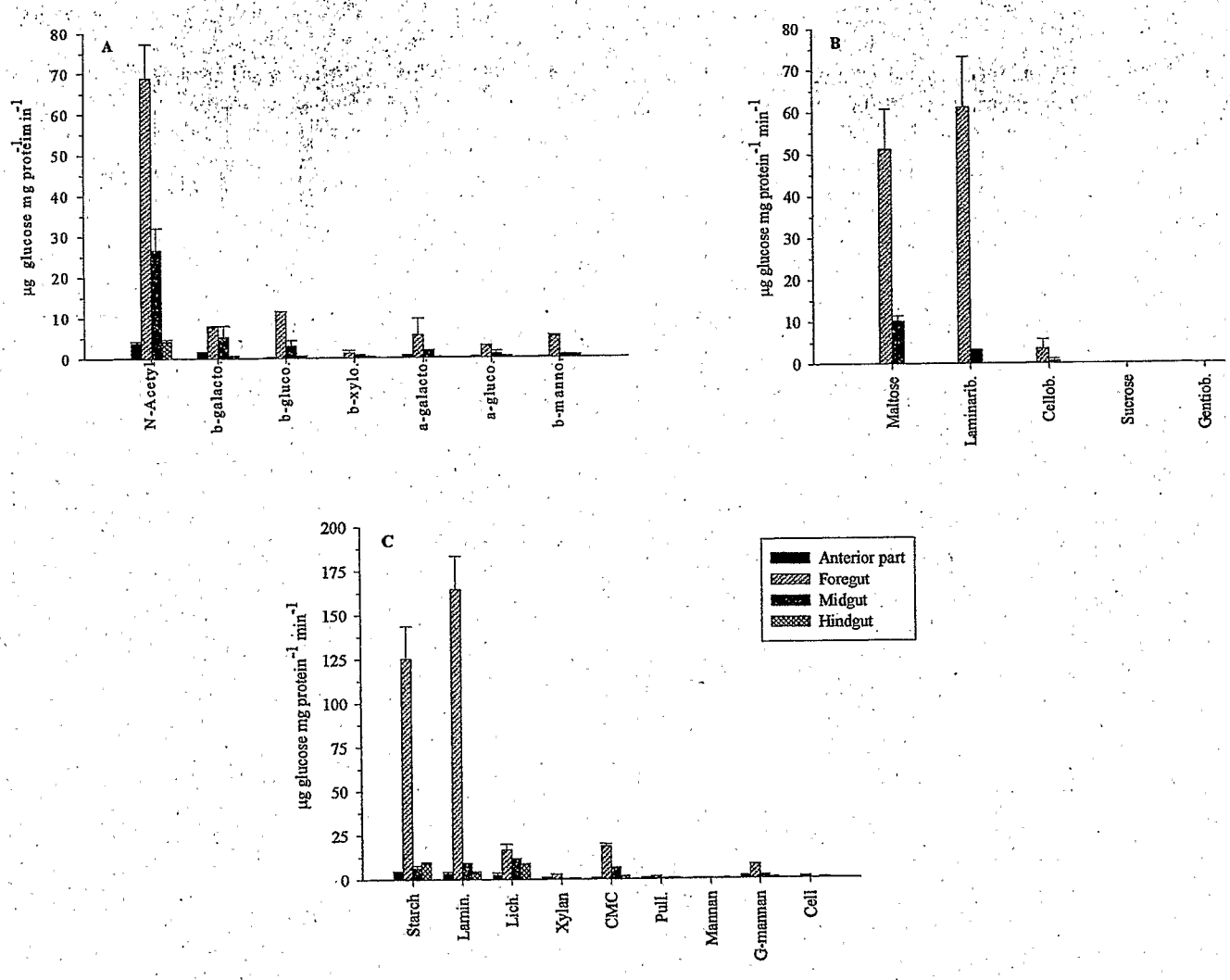

Fig. 1. Specific enzymatic activities (μg mg^{-1} min^{-1}) in the whole gut of *H. elisae*. (A) Heterosides; (B) Oligosaccharides; (C) Polysaccharides.

Table 1
Enzymatic activity (μg glucose mg protein^{-1} min^{-1}) in the tissues of earthworms at different cultural times

Cultured tissues	3 days of culture			5 days of culture			7 days of culture		
	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Heterosides									
N-Acetyl	2.04±0.04	1.66±0.06	0.10±0.02	0.80±0.09	0.54±0.04	0.20±0.02	1.07	0.41±0.07	0.10±0.02
β-Galactoside	0.76±0.05	0.09±0.04	0.03±0.02	0.30±0.05	0.23±0.05	0.11±0.04	0.44±0.19	0.08±0.02	0.06±0.03
β-Glucoside	0.55±0.02	0.08±0.07	0	0.11±0.07	0.05±0.02	0.02±0.02	0.31±0.06	0	0
β-Xyloside	0.15±0.01	0.02±0.02	0.02±0.01	0.02±0.02	0.06±0.01	0.03±0.01	0.03±0.03	0	0.02±0.01
α-Galactoside	0.64±0.04	0.04±0.01	0	0.22	0.04	0	0.70±0.04	0.01±0.01	0
α-Glucoside	0.04±0.01	0	0	0.02±0.01	0.01±0.01	0	0	0.01±0.01	0
β-Mannoside	0.01	0	0	0.01±0.01	0.01±0.01	0.01	0.06±0.02	0	0
Oligosaccharides									
Maltose	0.25±0.01	0.10±0.01	0	0.68±0.02	0	0	0.54±0.12	0	0
Laminaribiose	0	0	0	1.11±0.24	0	0	0	0	0
Cellobiose	0	0	0	0.12±0.07	0	0	0	0	0
Polysaccharides									
Starch	0.22±0.02	0.02±0.02	0.05±0.01	0.26±0.01	0.07±0.07	0.04±0.01	0.7±0.09	0.02±0.02	0.01±0.01
Laminarin	1.21±0.02	0.01±0.01	0.03	0.89±0.04	0.02	0.02±0.01	0.74±0.09	0.04±0.04	0
Lichenin	0.33±0.07	0	0.05±0.01	0	0.1±0.02	0	0.2±0.07	0.02±0.02	0
Xylan	0.13±0.08	0	0.05	0.06	0	0	0.02±0.02	0	0
CMC	0.43±0.02	0	0	0.23	0	0.03±0.03	0.11±0.03	0.03±0.03	0.01±0.01
Pullulan	0.07±0.01	0	0.02±0.01	0.03	0	0.01±0.01	0	0	0.02
Mannan	0	0	0	0	0	0	0	0	0
Galactomannan	0.03±0.03	0	0	0.03±0.03	0	0	0.01±0.01	0	0
Cellulose	0	0	0	0	0	0	0	0	0

Table 2
Total activity (μg glucose min^{-1}) in the culture medium at different cultural times

Culture medium	3 days of culture			5 days of culture			7 days of culture		
	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Heterosides									
N-Acetyl	1.29±0.19	1.66±0.50	0.19±0.05	0	1.75±0.3	18.40±2.24	1.33±0.04	1.13±0.06	0
β-Galactoside	0.53±0.09	0.03±0.03	0	10.89±1.93	0	0.37±0.12	0.12	0.44±0.06	0
β-Glucoside	0.44±0.00	0.38±0.06	0.34±0.09	36.71±0.62	0	0	0.22±0.03	0.59±0.03	0.12±0.06
β-Xyloside	0.31±0.06	0	0.19	0	0	0	0.09±0.09	0.25	0.22±0.09
α-Galactoside	0.05±0.02	0	0	0	0	0.09±0.06	0.16±0.04	0.01±0.01	0.04±0.04
α-Glucoside	0.03±0.03	0	0.01±0.01	0.67±0.43	1.17±0.1	0.13±0.07	0.02±0.02	0.03	0
β-Mannoside	0.04±0.01	0	0	0	0	0	0.07±0.01	0.01±0.01	0
Oligosaccharides									
Maltose	0	0	0	2.8	8.49±0.29	0	3.14±0.14	0	0
Laminaribiose	0	0	0	1.83±0.29	0	0	0.39	0	0
Cellobiose	0	0	0	3.47±0.29	0	0	2.03±0.29	0	0
Polysaccharides									
Starch	0.94±0.31	0.51±0.01	0.82±0.27	0	0	0	1.19±0.28	2.12±0.83	0.36±0.01
Laminarin	1.14±0.11	0.76±0.01	1.85±0.61	0	0	0	0.97±0.17	1.27±0.23	1.36±0.34
Lichenin	0.33±0.15	0.72±0.06	1.17±0.22	1.65±0.43	0	4.12±0.22	0.16±0.04	0.97±0.13	0.48±0.29
Xylan	1.13±0.26	0.98±0.04	1.47±0.23	0	0	0	0.26±0.10	1.52±0.31	0.75±0.10
CMC	1.64±0.03	0.84±0.03	1.76±0.24	0	0	0	1.20±0.05	1.85±0.24	0.94±0.62
Pullulan	0	0.14±0.07	1.17±0.06	11.08±0.95	0	0	0.04±0.04	0.47±0.11	0.07±0.07
Mannan	0	0	0	0	0	0	0	0	0
Galactomannan	0.34±0.02	0.13±0.01	0.29±0.21	0	0	0	0.15±0.02	0.95±0.20	0
Cellulose	0	0	0	0	0	0	0	0	0

the most degraded substratum was again *N*-acetylglucosamine. Among polysaccharides the breaking up of laminarin and starch was again important, but it was stronger for CMC. Laminarinase activity was stronger in 3 and 7-day cultures, but disappeared at the 5th day. Starch had the same behaviour as laminarin. In the culture medium both mannanase and cellulase activities were not detected. The activity on oligosaccharides appeared only at the 5th day of culture and generally was higher than in the cultured tissues.

4. Discussion

Results suggested that *H. elisae* has a wide glycolytic enzymatic system. In the whole gut enzyme activities were, generally, in the range observed in other endogeic geofagous earthworms such as *P. corethrurus* (Zhang et al., 1993), *M. anomala* and *Polypheretima elongata* (Lattaud et al., 1997a, b). The heterosidasic activity was much weaker than that of other invertebrates such as termites (Rouland, 1986) and larvae of some forest Diptera (Deleporte and Rouland, 1991) but similar to that of the earthworm species cited above. Oligosaccharide enzyme activities were similar, except for laminaribiase, having *H. elisae* a more important activity than the other earthworm species. Considering polysaccharidases, starch and laminarin enzymes were important, but the other polysaccharides were hardly degraded, whilst in the cited earthworm species some activity was always found. Starch was less hydrolysed in *H. elisae* than in *P. elongata*, but more than in *M. anomala* and other invertebrates such as some Diptera larvae (Deleporte and Charrier, 1996), and enchytraeids (Šustr and Chalupský, 1996) and even some termites (Rouland, 1986). Laminarinase activity was much more important in *H. elisae* than in any of the other cited earthworm species. The especially weak activity on cellulose, hemicelluloses, cellobiose and most heterosides is in accordance with the ecological requirements of *H. elisae*, since it is an endogeic oligohumic species that feeds on soil low in organic matter. Loquet and Vinceslas (1987), Urbášek (1990) and Urbášek and Pizl (1991) observed that the cellulase activities in the gut of endogeic earthworms were lower than in epigeic species.

The analysis of enzyme activities is useful to determine the substrates on which earthworms feed (Rodière, 1994). *N*-acetylglucosamine is a characteristic component of fungal cell walls, and laminarin, laminaribiose and starch are characteristic from roots. The activities on all these substrates were high in the gut of *H. elisae*, suggesting that it may feed on fungi, small decaying roots and root exudates. Barois (1987) noted that the number of fungal propagules decreased through earthworm gut transit. *N*-acetylglucosaminase and laminarinase were also found to be abundant in the gut of the tropical endogeic earthworms *P. corethrurus* (Zhang et al., 1993), *M. anomala* and *P. elongata* (Lattaud et al., 1997a, b). This fact is very important to understand how *H. elisae* can survive in such a poor soil. CMC, cellobiose and xylan are characteristic of dead litter. The enzymatic activity on CMC was low and the activities on both cellobiose and xylan were very low, thus these substrates do not seem to be food for *H. elisae*. Similar values of enzyme activities for these substrates were found in the tropical endogeic *M. anomala* and *P. corethrurus* (Lattaud et al., 1997a, b). Sucrose is usually found in fresh litter but there was no activity on this substrate in the gut of *H. elisae*, which is consistent with this species not feeding on fresh material.

H. elisae did not produce any oligosaccharide enzymes degrading in the hindgut. This result had not been observed in any of the studied species, suggesting that *H. elisae* breaks up these substrates in the first part of the gut and is able to uptake resulting residues in one or two of the other parts.

Comparing our results of enzymatic activities in the intestinal tissues with those from Zhang et al. (1993) and Lattaud et al. (1997a, b), the activities in the gut walls of *H. elisae* were very weak. This suggests that the absence of microorganisms leads to an important decrease (but not lack) of the enzymatic capacity of *H. elisae*. The absence of cellulase and mannanase activities suggests that *H. elisae* cannot produce these enzymes, so it would need ingested microorganisms to digest corresponding substrates, which implies mutualistic associations. This result agrees with Lavelle et al. (1995) who noted that most of the soil invertebrates did not seem to have a suitable equipment for cellulose degradation, although cellulases have been found in the gut contents of some groups, seemingly produced by ingested micro-organisms. Trigo et al. (1999), analysing the amount of intestinal mucus in different earthworm species, reported that this mutualistic digestion system was important in *H. elisae*. Nevertheless, the low lysis of cellulose in the whole gut (when microflora is present) suggests that the degrading capability for this substrate is insignificant in any case. The same fact happened for mannanase, so the importance of the mutualistic system is not so clear in this species and more studies will be necessary for a better understanding of this digestion system.

Acknowledgements

Thanks to the chemical Technician S. Sylvain from ORSTOM, for his friendly technical support.

References

Álvarez, J., 1977. El género *Hormogaster* en España. Publicaciones del Centro Pirenaico de Biologia Experimental 9, 27–35.

Barois, I., 1987. Interactions entre les Vers de Terre (Oligochaeta) tropicaux géophages et la microflore pour l'exploitation de la matiére organic du sol. Thèse Université Paris VI.

Brown, G.G., 1995. How do earthworms affect microfloral and faunal community diversity? Plant and Soil 170, 209–231.

Deleporte, S., Charrier, M., 1996. Comparison of digestive carbohydrases between two forest sciarid (Diptera: Sciaridae) larvae in relation to their ecology. Pedobiologia 40, 193–200.

Deleporte, S., Rouland, C., 1991. Étude préliminaire de l'equipement digestif osidasique de *Bradysia confinis* (Diptera, Sciaridae): implications dans la dégradation de la matière organique. Comptes Rendues de l'Académie des Sciences de Paris 312 (III), 165–170.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman and Hall, London.

Karsten, G.R., Drake, H.L., 1995. Comparative assessment of the aerobic and anaerobic microfloras of earthworm guts and forest soils. Applied Environmental Microbiology 61, 1039–1044.

Lattaud, C., Locati, S., Mora, P., Rouland, C., 1997a. Origin and activities of glycolytic enzymes in the gut of the tropical geophagous earthworm *Millsonia anomala* from Lamto (Côte d'Ivoire). Pedobiologia 41, 242–251.

Lattaud, C., Zhang, B.G., Locati, S., Rouland, C., Lavelle, P., 1997b. Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (Megascolecidae). Soil Biology and Biochemistry 29, 335–339.

Lavelle, P., 1983. The structure of earthworm communities. In: Satchell, J.E. (Ed.), Earthworm Ecology: from Darwin to Vermiculture. Chapman & Hall, London, pp. 449–466.

Lavelle, P., Lattaud, C., Trigo, D., Barois, I., 1995. Mutualism and biodiversity in soils. In: The significance and Regulation of Soil Biodiversity. Collins H.P., Robertson G.P.H., Klug M.J. (Eds.), The Netherlands, pp. 23–33.

Lavelle, P., Zaidi, Z., Schaefer, R., 1983. Interactions between earthworms, soil organic matter and microflora in an African savanna soil. In: Lebrun, P., Andre, A.M., De Medts, A., Gregoire-Wibo, C., Wauthy, G. (Eds.), New Trends in Soil Biology. Dieu-Brichard, Louvain-La-Neuve, Belgium, pp. 253–259.

Laverack, M.S., 1963. The physiology of earthworms. In: Kerkut, A.G. (Ed.), International Series Monograph on Pure and Applied Biology. Pergamon Press, Oxford.

Loquet, M., Vinceslas, M., 1987. Cellulolyse et lignilolyse liées au tube digestif d'*Eisenia fetida andrei* Bouché. Revue d'Ecologie et Biologie du Sol 24, 549–558.

Nelson, N., 1944. Photometric adaptation of Somogyi method for determination of glucose. Journal of Biology and Chemistry 153, 275–380.

Parle, J.N., 1963. Micro-organisms in the intestine of earthworms. Journal of General Microbiology 31, 1–11.

Rodière, E., 1994. Etude de l'activité enzymatique glucidique d'un ver de terre tropical endogeé *Polypheretima elongata*. Maîtrise de Biologie des Organisms et des Populations. Université Paris VI.

Rouland, C., 1986. Contribution à l'étude des osidases digestives de plusieurs espèces de Termites africains. Thèse Université Paris VI.

Sedmark, J.J., Grossberg, S.E., 1977. A rapid, sensitive and versatile assay for protein using coomasie brilliant blue G 250. Annals of Biochemistry 79, 544–552.

Somogyi, M., 1945. Determination of blood sugar. Journal of Biology and Chemistry 160, 61–68.

Šustr, V., Chalupský, J., 1996. Activity of digestive enzymes in two species of potworms (Oligochaeta, Enchytraeidae). Pedobiologia 40, 255–259.

Trigo, D., Barois, I., Garvín, M.H., Huerta, E., Irisson, S., Lavelle, P., 1999. Mutualism between earthworms and soil microflora. Pedobiologia 43, 866–873.

Urbášek, F., 1990. Cellulase activity in the gut of some earthworms. Revue d'Ecologie et Biologie du Sol 27, 21–28.

Urbášek, F., Pizl, V., 1991. Activity of digestive enzymes in the gut of five earthworm species (Oligochaeta; Lumbricidae). Revue d'Ecologie et Biologie du Sol 28, 461–468.

Werner, W., Rey, H.G., Wiellinger, R.H., 1970. Properties of a new chromogen for determination of glucose in blood according to GOD/POD method. Frezenius. Annals of Chemistry 252, 224–228.

Zhang, B.G., Rouland, C., Lattaud, C., Lavelle, P., 1993. Activity and origin of digestive enzymes in gut of the tropical earthworm *Pontoscolex corethrurus*. European Journal of Soil Biology 29, 7–11.

Soil Biology & Biochemistry

ISSN 0038-0717

IUSS-UISS-IBU-UICS

Vol. 32 No. 7
July 2000
Editor-in-Chief J S WAID