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The diversity of digestive systems in tropical geophagous earthworms

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Abstract

Some of the enzymes found in the gut contents of endogeic geophagous earthworms are produced by ingested microflora. This study compares the origin and activities of glucosidic enzymes present in the gut contents of adult *Polypheretima elongata* from Sainte Anne (Martinique), *Pontoscolex corethrurus* from Palma Sola, Veracruz (Mexico) and *Millsonia anomala* from Lamto (Côte d'Ivoire). Substrates characteristic of plant material were used to compare enzymatic capabilities of gut contents and isolated gut tissues. All substrates tested were digested which indicates that all three species may use root and fungal substrates available in soils. In vitro tissue culture of *P. elongata* produced all the glucosidic enzymes unlike cultures of the other two species which required microbial activity to synthetize mannanase and cellulase; moreover, glucosidic activities measured in *P. elongata* were higher than those found in *P. corethrurus* and *M. anomala*. © 1998 Elsevier Science B.V.

Keywords: Earthworms; Glucosidic digestive enzymes; *Pontoscolex corethrurus*; *Polypheretima elongata*; *Millsonia anomala*; In vitro intestinal tissue culture; Ingested microflora

1. Introduction

Endogeic earthworms are a dominant component of soil animal communities in humid tropical savannas. Their high rates of ingestion and specific digestive processes allow them to feed on soils which are poor in organic matter and to enlarge their ecological niche (Lavelle, 1983). Earthworms play an active part in soil organic matter dynamics and nutrient turnover. The intestinal mucus of these earthworms contains easily metabolizable components that disappear rapidly during gut transit and trigger a flush of microbial activity and the subsequent digestion of organic matter observed in the gut (Barois and Lavelle, 1986). Endogeic earthworms seem to digest soil organic matter through a mutualistic earthworm–microflora digestion system and the production of the intestinal mucus is a key process in earthworm digestion (Lavelle et al., 1995). In fact, the majority of soil invertebrates do not seem to possess enzymes capable of digesting cellulose, lignin, tannin and humic complexes, which are the most available resources in soil, directly. Although enzymes such as cellulase are present in the gut contents of some soil invertebrates, they seem to be produced by ingested micro-organisms rather than by the invertebrate itself (Loquet and Vinceslas, 1987; Urbasek, 1990).

The aim of our experiments was to identify glucosidic enzymatic activities in the gut of some tropical endogeic earthworms (Megascolecidae) and to deter-

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mine whether these enzymes were produced by the worms themselves or by the ingested soil microflora. These activities were measured in the gut (wall and contents) in order to demonstrate the origin of these enzymes, a tissue culture of gut walls was achieved and enzymatic activities were evaluated both in the cultured tissues and in the culture media.

2. Materials and methods

Adult earthworms *Pontoscolex corethrurus* Müll. from Palma Sola, Veracruz (Mexico), *Polypheretima elongata* Perr. from Sainte Anne (Martinique) and *Millsonia anomala* Omo. collected at Lamto (Côte d'Ivoire) were maintained in their original soil in a control chamber at 28°C.

2.1. Preparation of enzyme solutions

Earthworms were dissected in icy physiological Holtfreter solution (8.77‰ NaCl, w/v). The gut, comprising wall tissues and contents, was separated into two parts. Portions of each part of the gut from between seven and ten earthworms, depending on the species, were pooled, homogenized and dialised as described by Zhang et al. (1993). The solution obtained was used as the source of the enzyme.

For the in vitro culture of intestinal tissues, foregut, midgut and hindgut, samples were placed into issue culture dishes in a culture medium described by Lattaud et al. (1997a, b). The dishes were agitated for 3 to 7 days in a control chamber at 28°C. At the end of the culture period, the cultured gut wall tissues and the culture media were frozen. Enzyme solutions from tissues and culture media were prepared following Lattaud et al. (1997b).

2.2. Enzyme activity assay

A large range of synthetic oligosaccharide, heteroside and polysaccharide substrates, selected mainly because of their plant origin, were tested, and glucosidic activities in the gut were assayed using the methods described by Zhang et al. (1993). Specific glucosidic activities in the gut and tissue culture were expressed as μg glucose released per mg of protein per minute; for the culture media the glucosidic activities were expressed as the total activity (μg glucose per minute).

3. Results

3.1. Specific glucosidic activities in the gut

All the substrates tested could be digested by the three earthworm species studied, which indicates that these earthworms were able to degrade the root and fungal substrates available in soils (Tables 1–3). Enzymatic glucosidic release was almost non-existent in the first part of their gut which included the pharynx, oesophagus, crop and gizzard. However, glucosidic activities were higher in the gut of *P. elongata* and *M. anomala* than in the gut of *P. corethrurus*. Among the oligosaccharides tested, maltose was the most readily degraded substrate. This was mainly in the foregut, midgut and hindgut of *P. elongata* and *M. anomala*. *N*-acetylglucosaminase had the highest activity of the heterosidases detected in each species, with maximum activity localized in the foregut of *M. anomala*, in the foregut and midgut of *P. corethrurus* and in the hindgut of *P. elongata*. All the polysaccharides were broken down to varying extents: very high specific activity was detected on starch in the three parts of the gut of *P. elongata* and in the foregut and midgut of *P. corethrurus*; a high laminarinase-specific activity was also observed in the foregut of *P. elongata* and *M. anomala*. It is worth noting that cellulase- and mannanase-specific activities were present in the gut of the three species although both cellulose and mannan were weakly degraded.

3.2. Specific activities in tissue culture

The quantities of cultured gut wall tissues and culture media recovered for *P. elongata* and *M. anomala* were sufficient to allow quantitative analysis of the glucosidic enzymatic activities but not enough cultured intestinal tissues were available for quantitative studies of *P. corethrurus*. The maximum glucosidic activities in tissue culture medium of *P. corethrurus* and *P. elongata* were obtained after 3 days of culture and the intestinal wall tissues of *M. anomala* showed highest glucosidic activities after 3, 5 or 7 days,

Table 1
Enzyme activities in the gut of *Pontoscolex corethrurus*[a], in the intestinal wall tissues and culture media after 3 days of culture

Pontoscolex Corethrurus	S.A. in the gut				S.A.in tissue culture			T.A. in culture media		
	p+o+c+g	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Oligosaccharides										
Maltose	3.64±0.61	16.27±2.53	13.7±180	3.65±0.63	++	++	++	+	+	+
Laminaribiose	0	4.89±0.02	3.11±0.02	0.41						
Heterosides										
α-Glucoside	0.3±0.01	1.48±0.17	1.52±0.11	0.38±0.09						
β-Glucoside	0.35±0.01	1.32±0.02	1.95±0.02	1.3±0.01						
N-acetyl	2.98±0.94	56.11±1.67	70.58±9.18	11.4±1.69	++	++	++	+	+	+
Polysaccharides										
Starch	2.82±0.7	26.52±3.51	26.48±2.42	5.85±0.05	+	+	+	+	+	+
Cellulose	0.67	1.89±0.02	1.99±0.01	1.85±0.01	0	0	0	0	0	0
Laminaran	1.35±0.01	12.25±1.71	8.84±0.88	2.28±0.74	+	+	+	+	+	+
Mannan	0	7.88±1.72	9.74±1.34	1.77±0.04	0	0	0	0	0	0
Glucomannan	1.85±0.02	12±0.02	8.22±0.04	3.35±0.02						
Lichenin	1.35±0.02	4.28±0.04	3.77±0.09	2.75±0.02						

[a] Data from Zhang et al. (1993).
S.A. in the gut and tissue culture; specific glucosidic activity expressed as μg glucose. mg^{-1} protein. mn^{-1}.
T.A. in culture media: total glucosidic activity expressed as μg glucose. mn^{-1}.
o, non-detectable glucosidic activity; +, weak glucosidic activity; ++, high glucosidic activity; p, pharynx; o, oesophagus; c, crop; g, gizzard.
Cellulose and mannan were not broken down in the cultured tissues of gut wall of *P. corethrurus*.

depending on the substrate. Activities in the first part of the gut were nearly non-existent and only specific activities in the foregut, midgut and hindgut were evaluated. The glucosidic activities were higher in the tissue culture of *P. elongata* than in *M. anomala*. In each earthworm studied, the main oligosaccharidase-specific activities were detected on maltose. This was mainly after 3 and 7 days of culture in all three parts of the gut of *P. elongata* and in the hindgut of *M. anomala*. These activities were similar to those of the gut (walls and contents) for *P. elongata* and were approximately half those of the gut of *M. anomala*. An important maltase-specific activity was also observed in the cultured tissues of *P. corethrurus*. A high level of laminaribiase activity was released only from the foregut of *P. elongata*. The main heterosidase-specific activity was observed on *N*-acetylglucosamine in the foregut of *P. elongata* and *P. corethrurus* and in the foregut of *M. anomala* respectively after 3 and 5 days of culture; for *P. elongata* this activity remained higher in the tissue culture than in the gut. The obvious polysaccharidase activities observed were those of amylase, in all three parts of the cultured gut wall tissues of *P. elongata*. These activities were about half those of the foregut and midgut. Polysaccharidase activities were very weak in the tissue culture of the other two species. Among the other polysaccharides, laminaran and lichenin were very weakly degraded in the cultured intestinal tissues. It is worth noting that cellulose and mannan were broken down, except in the tissue culture of *M. anomala* and *P. corethrurus*.

3.3. Total activity in the culture medium

Culture medium is full of exogenous proteins and a specific activity cannot be related to them; instead enzymatic activities were expressed as total activity (μg of reducing sugars. min^{-1}) of 2.75 ml and 2.2 ml of culture medium for *P. elongata* and *M. anomala* respectively. For *P. corethrurus* the small quantities of cultured gut wall tissues and culture media did not allow quantitative determination of total activities in the culture media.

Among the oligosaccharides studied, high total activities on saccharose only appeared in the foregut,

Table 2
Enzyme activities in the gut of Polypheretima elongata [b], in the intestinal wall tissues and culture media after 3 days of culture

Polypheretima elongata

	S.A. in the gut				S.A. in tissue culture			T.A. in culture media		
	P+O+c+g	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Oligosachharides										
Saccharose	0.66±0.06	37.06±3.56	26.79±1.44	26.1±0.65	12.22±4.07	28.11±3.86	25.68±1.97	1.7±0.42	5.84±1.03	
Maltose	3.63±0.61	79.43±4.5	55.89±4.54	63.51±4.61	82.59±0.27	60.91±0.27	61.23±5.05	4.79±0.97	13.51±2.21	
Laminaribiose	0.18±0.02	7.72±1.55	4.67±1.42	4.62±1.3	62.22±0.74	17.92±0.66	10.97±1.32	5.5±1.2	4.28±1.12	1.7±0.06
Heterosides										
α-Glucoside	0.27±0.15	4.86±0.95	3.64±0.88	7.89±0.69	12.3±0.55	3.9±0.01	5.5±0.05	0.26±0.04	0	0
β-Glucoside	0.62±0.09	7.46±0.83	5.77±1.13	0.11±0.01	43.33±6.67	9.92±0.49	0	3.18±0.87	1.04±0.32	0
N-acetyl	2.77±0.68	26.33±4.85	14.97±0.82	46.5±1.5	167.5±2.5	3.3±7.17	14.28±0.53	15.52±1.48	1.72±0.27	0.8±0.04
Polysaccharides										
Starch	1.75±0.31	197.73±7.72	188.72±3.68	90.65±4.77	88±1.11	80±2.36	87.24±0.88	0.34±0.08	0	2.52±0.74
Cellulose	0.75±0.03	6.27±1.13	8.58±1.62	1.27±0.02	0.04±0.01	0.54±0.01	0.26±0.10	0.44±0.08	0.42±0.03	0
Laminaran	0.28±0.04	40.93±1.46	19.35±0.89	10.46±0.49	1.55±0.01	2.64±0.08	0.36±0.02	0	0	0
Mannan	0.74±0.03	2.96±0.09	4.11±0.22	1.34±0.01	0.7±0.08	0.99±0.01	0.27	0.29±0.03	0.46±0.04	0.071
Lichenin	0.66±0.03	6.63±1.8	6.05±1.22	26.27±0.55	12.5±1.05	5.91±1.77	3.85±0.88	0	0	0

[b] Data from Lattaud et al. (1997a)

S.A. in the gut and tissue culture; specific glucosidic activity expressed as μg glucose. mg^{-1} protein. mn^{-1}.

T.A. in culture media: total glucosidic activity expresed as μg glucose. mn^{-1}.

o, non-detectable glucosidic activity; +, weak glucosidic activity; ++, high glucosidic activity; p, pharynx; o, oesophagus; c, crop; g, gizzard.

Cellulose and mannan were broken down in the cultured tissues of gut wall of *P. elongata*.

Table 3
Enzyme activities in the gut of Millsonia anomala [c] after 7 days of culture. Mean of two independent assays ± standard error

Millsonia anomala	S.A. in the gut				S.A. in tissue culture			T.A. in culture media		
	p+o+c+g	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Oligosaccharides										
Saccharose	0.29±0.01	0.31±0.02	10.87±1.01	9.18±2.19	6.56±0.19	19.12±3.62	34.06±7.09	26.19±0.97	27.94±1.24	26.62±0.77
Maltose	2.02±0.12	48.64±4.55	47.41±4.46	30.39±3.24	39.02±3.25	25.88±0.78	95.09±4.25	0.04	0	0
Laminaribiose	1.77±0.08	13.51±2.02	16.60±2.14	4,27±2.14	19.24±2.43	30.10±2.84	19.16±2.12	0.04±0.01	0.19±0.03	0.04
Heterosides										
α-Glucoside	0.81±0.65	11.84±3.57	1.19±0.35	1.46±0.21	0.93±0.05	0	0	0.11±0.01	0.029	0.06±0.02
β-Glucoside	0.92±0.68	7.86±0.39	15.77±3.24	11.60±1.44	0.29±0.04	1.73±0.07	4.80±0.56	0.10±0.01	0	0.15±0.01
N-acetyl	2.63±0.98	87.39±9.01	18.62±3.51	19.71±3.25	16.87±1.06	8.18±0.29	9.02±0.26	0.02±0.01	0.05±0.01	0.06±0.02
Polysaccharides										
Starch	0.36±0.01	0.99±0.05	12.54±0.35	12.11±3.51	0.36±0.03	1.76±0.14	0.92±0.06	0.96±0.02	0.25±0.03	0.06±0.02
Cellulose	0.28±0.16	0.49±0.01	1.46±0.23	0	0	0	0	0	0	0
Laminaran	0.88±0.03	38.46±3.21	7.49±3.24	1.84±0.26	7.09±1.70	9.71±0.74	2.45±0.21	0.1	0.02	0
Mannan	0	5.60±0.88	3.93±0.93	1.62±0.36	0	0	0	0	0	0
Lichenin	0.87±0.02	1.50±0.64	1.74±0.23	0.77±0.06	1.93±0.42	14.49±0.75	0	0.97±0.16	0.57±0.16	0.57±0.16

[c] Data from Lattaud et al. (1997b).
S.A. in the gut and tissue culture; specific glucosidic activity expressed as µg glucose. mg^{-1} protein. mn^{-1}.
T.A. in culture media: total glucosidic activity expresed as µg glucose. mn^{-1}.
o, non-detectable glucosidic activity; +, weak glucosidic activity; ++, high glucosidic activity; p, pharynx; o, oesophagus; c, crop; g, gizzard.
Cellulose and mannan were not broken down in the cultured tissues of gut wall of *M. anomala*.

midgut and hindgut culture media of *M. anomala* after 7 days and these activities reached values similar to those detected in the cultured midgut and hindgut. Total activities on saccharose and laminaribiose were practically non-existent after 3 or 5 days of culture. A moderate total activity on laminaribiose was also observed in the midgut and hindgut culture media of *P. elongata*. The results on the heterosides tested showed only a moderate total activity on *N*-acetylglucosamine in the foregut culture media of *P. elongata*. For polysaccharides, the activities on starch, laminaran and lichenin, although weak, were generally present on both media and cultured tissues of the three species, except for *P. elongata*. Weak total activities on cellulose and mannan were present on both media and cultured tissues of *P. elongata*, but they were missing on those of *P. corethrurus* and *M. anomala*.

4. Discussion

This study demonstrated that these earthworms possessed a relatively complete glucosidic enzymatic system. However, the enzyme activities are rather weak compared to those of other invertebrates such as the snail *Helix aspersa* (Charrier and Rouland, 1992) and fungus-growing and xylophagous termites (Rouland et al., 1991). These activities can be compared to those detected in soil-feeding termites which are distinguished by their low oxidase content (Rouland, 1986). The earthworms studied feed on litter debris and soils poor in organic matter, which is consistent with the rather weak specific glucosidic activities detected in their gut. A large range of glucosidic substrates, characteristic of plant material, was broken down, which indicates that all three species may degrade root, litter and fungal substrates available in soils. In particular, they show a certain specific activity on cellulose and hemicellulose and therefore, they are likely to use most of the vegetal components in the soil for their nutrition. All the specific glucosidic activities detected in the gut and the intestinal tissue culture were higher in *P. elongata* than in *M. anomala*, which in turn showed higher activities than *P. corethrurus*. Maltose, *N*-acetylglucosamine, starch and laminaran were the substrates most efficiently hydrolysed on both gut and cultured tissues of the three species. However, glucosidic activities in the gut of *M. anomala* indicate that this earthworm can hydrolyse chitin sub-units which constitute the fungal cell wall and starch, a root substrate, to glucose and is thus is likely to feed on fungi and roots available in soils. Amylase- and maltase-specific glucosidic activities were higher in the gut of *P. elongata* than in *M. anomala*, which suggests that *P. elongata* feeds mainly on root substrates.

Gut wall tissue culture showed that *M. anomala*, like *P. corethrurus*, requires the digestive capacities of ingested soil microflora in order to degrade cellulose and mannan, while *P. elongata* can synthetize all its required extra- and intracellular enzymes. Urbasek (1990) demonstrated that, among the epigeic and endogeic earthworm species studied, only *Lumbricus rubellus* depended on symbiotic cellulosic microflora and was able to degrade cellulose. In nature, few organisms possess enzymes which can to hydrolyse cellulose and mannan, the main plant constituents, and they make use, like *P. corethrurus* and *M. anomala*, either of ingested bacteria which show cellulolytic and mannanase activities, or of symbiotic bacteria in order to degrade the insoluble substrates.

High total activity on saccharose and low activities on oligosaccharides, heterosides and polysaccharides were detected for the *M. anomala* culture media. All the glucosidases released were extracellular enzymes. Gut wall tissues of *P. elongata*, cultured for 3 days, secreted extracellular oligosaccharidases and heterosidases and from the polysaccharidases, the only extracellular enzymes released, were amylases, cellulases and mannanases, while laminarinases and licheninases were never detected in the media. The absence of laminarinases and licheninases in the culture media suggests that their secretion is induced.

These results suggest that geophagous endogeic earthworms display rather variable adaptative characters which are undoubtedly linked to the different ecological categories and niches.

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